STUDIES IN THE LIFE-HISTORY AND TAXONOMY OF THE GENUS ENTEROMORPHA

A thesis submitted for the Degree

of

Master of Science

and Honours

by

David J. Rawlence

University of Canterbury

New Zealand

1966

"I do not know what I may appear to the world, but to myself I seem to have been only like a boy playing on the seashore, and diverting myself in now and then finding a smoother pebble or a prettier shell than ordinary, whilst the great ocean of truth lay all undiscovered before me."

Newton

CONTENTS

INTRODUCTION

(i) INTRODUCTION AND STATEMENT OF OBJECTIVES

The genus Enteromorpha is usually placed in the family Ulvaceae (Bliding, 1963; Taylor, 1937, 1960; Chapman, 1956). This family includes those green algae with flat or tubular thalli, and cells with one or two lateral chromatophores and a single pyrenoid. The asexual spore-producing and sexual gamete-producing generations are morphologically identical (Bliding, 1963 P.41).

Enteromorpha includes the branched or unbranched monostromatic members of the Ulvaceae which are hollow and tubular.

A variety of life histories* have been described for the Genus. These include monomorphic diplontic - Enteromorpha intestinalis var asexualis (Bliding, 1963), Enteromorpha biflagellata (Bliding, 1944); Monomorphic diplohaplontic - several species including Enteromorpha ramulosa (Hartmann, 1929), Enteromorpha intestinalis (Kylin, 1930 a.; Bliding, 1948 a.); Monomorphic haplontic Enteromorpha stipitata P. Dangeard var linzoides nov. var. (Bliding, 1960).

No dimorphic diplohaplontic life histories have as yet been described for any member of the Ulvaceae. There have, however, been indications of their existence in some genera.

Pocock (1961) investigated this possibility in Letterstedtia. Chapman (1956) discussed the possible alternation of a branched and

---

* Drew (1955) terminology.

unbranched generation in Enteromorpha australiensis unsupported to date by experimental evidence.

The main object of this thesis was to study any local Enteromorpha population which appeared to have a dimorphic diplohaplontic life history. The writer, found evidence of such a life history in an Enteromorpha population in the Motunau River, North Canterbury. This thesis details a series of observations and experiments with the object of confirming this observation.

During preliminary experimental work, however, several additional problems emerged. These required investigation before the original objective could become meaningful. One of those problems was the classification of the populations studied.

Early attempts to classify the Motunau River population proved difficult. The characters usually considered to be of greatest taxonomic importance were found to vary considerably within the population. As a result a dichotomous key would not place a plant unambiguously into a single species. It was felt that a greater knowledge of the variation in the diagnostic characters was necessary before any species could be accurately identified.

In order to elucidate the systematic position as well as the life history of the Motunau Enteromorpha population it was necessary to establish cultures. Fertile plants had to be collected and zooid release effected in the laboratory. Extreme difficulty was experienced in both these respects. It was difficult to find any fertile plants and even more so to effect zooid release. Emphasis was therefore placed

upon periodicity observations and the conditions influencing zooid release in the laboratory and natural environment.

Once the desired cultures were established, the extreme variability of orthodox taxonomic criteria led to the search for others, and the possibility of using embryology as a taxonomic criterion was investigated. Here additional problems emerged. Plants of the same age were observed to have widely different embryo form. This situation was found in a number of populations.

The objectives of this thesis therefore became to study (1) the natural variation of selected taxonomic characters, (2) zooid release in the natural environment and in the laboratory, (3) the variation in embryology of several populations, and (4) to determine the type of life history possessed by an Enteromorpha population growing in the Motunau River, North Canterbury.

The subsequent chapters follow this order.

(ii) THE SIGNIFICANCE OF VARIATION IN TAXONOMIC CHARACTERS: GENERAL

Many of the problems encountered in these chapters, caused by character variation, apply to other populations of plants and animals. It is appropriate therefore to examine the approach to variation within the broad framework of modern knowledge of evolution and systematics.

The discussion may conveniently begin with the species, which was the level at which the present study was made. The studies of Clausen et al (1939), Mayr (1942), Dobzhansky (1941) and Huxley (1940) emphasise the facts that (1) each species varies morphologically, physiologically and genetically within itself and (2) that species

usually differ from one another by gaps in the ranges of variation of these features. Therefore it is not surprising that subspecies, varieties and species differ from one another only in degree, and not kind of taxonomic characters.

E.g. in Potentilla glandulosa the same type of characters are used to separate ecotypes and species (Stebbins, 1950).

Statements that some divergence in morphological and physiological characters accompanies species formation are gross simplifications. Closely similar species are frequently separated by weak isolating mechanisms and hybridization can be effected relatively easily (Stebbins, 1950). In other groups, widely divergent morphological forms grade into each other by a series of intermediates, and conversely, sharp distinctions may sometimes be drawn between species externally very similar (Stebbins, 1950).

In summary, the differences between species are frequently fragile. However, in any population, some characters at least will be constant, and others highly variable. In order to separate different species, it is necessary to differentiate between the two by 'weighting'. According to Davis and Heywood (1963) there are four methods of doing this.

(1) Where there is a large number of characters, one or a few may be described, simply for convenience.

(2) A character may be selected because it shows the highest correlation with others. This 'correlation weighting' is probably the only method which is theoretically sound (Davis and Heywood).

(3) In other cases a character may be selected because it is thought to have phylogenetic or other significance.

(4) When all other characters have been rejected, one or few may be left, which therefore have to be used - this is called residual weighting.

In addition to reproductive isolation, species should be separable with properly weighted qualitative and quantitative characters. There should be a difference in frequency of qualitative characters between two species e.g. the percentage of individuals with character A may be 0 - 20% in one and 80 - 100% in the other group, and a difference in modal values of quantitative characters i.e. an absence of values intermediate between two modal figures, each characteristic of a separate species.

The current situation in Enteromorpha.

Bliding has employed reproductive barriers to delimit species and obtain information on their more stable taxonomic criteria in culture, while other workers in taxonomy have been largely concerned with describing a few specimens of each type.

The writer found that existing records enabled him to classify only one South Island population. No combination of qualitative characters (gross morphology, chloroplast morphology, chloroplast and thallus colour) or quantitative characters (number of pyrenoids per cell, cell size and arrangement, and width of mucilage envelope) proved satisfactory. This is apparently caused by too little attention being given to the range of variation in entire populations, and to the proper weighting of characters, a situation which is best summed up in the

words of Stebbins (1950) 'some ..... base their philosophy on ..... if two things are different, they should be described as different species, ... these workers never form a clear conception of what they mean by "things" or "different".

The object of the chapters dealing with taxonomic character variation was only to justify the writer's reluctance to classify the South Island Enteromorpha populations collected for this study. However, during the course of these investigations, correlation was detected between cell division and convolution, and between convolution and large size plants. Attention was also given to the problem of character weighting. The degree of reliability which could be placed upon chloroplast morphology, the number of pyrenoids per cell, and in a later section, embryology, was investigated. These studies serve to emphasise the gaps in our taxonomic knowledge, especially the variation of characters within populations, which will have to be bridged before any real progress can be made on the taxonomy of the Genus.

The present position is admirably summed up in the following paragraph from Davis and Heywood (P.79, 1963).

"All available material should be used when classifying a group. Characters which appeared to provide good differentiae when the group was first described may no longer hold when more material is available; this applies particularly to specific and generic concepts. Additional specimens frequently break down the differences used to delimit species in the pioneer phase of classification. New species described by various authors accumulate in genera originally based on far fewer species, so that a genus may become too broad and its content too heterogeneous for maintenance as a single genus; on the other hand,

what were initially treated as two genera may have to be merged into one."

CHAPTER ONE - THE VARIATION IN TAXONOMIC CHARACTERS

BACKGROUND

The initial work for this thesis consisted of the location of a suitable Enteromorpha population for study. Collections were made from the following localities:- (Refer Figure 1)

(1) Heathcote River Estuary, Sumner, Christchurch

(2) Motunau River Estuary, Motunau, North Canterbury

(3) Taylor's Mistake, Banks Peninsula

(4) Seal Point, Kaikoura, North Canterbury

(5) Okarito Lagoon, West Coast

(6) Nine Mile Creek, West Coast

(7) Coastline one mile north of Nine Mile Creek, West Coast

(8) Mouth of Punakaiki River, West Coast

(9) Woodpecker Bay, West Coast

(10) Little Papanui, Otago Peninsula

(11) Pakawa River, N.W. Nelson

Careful observations at Motunau during the period January - December 1964 indicated that this population might possess a dimorphic alternation of generations. This had not previously been established for any Enteromorpha species.

Intensive investigations therefore began at Motunau in January 1965, with a view to confirming the existence of a dimorphic diplohaplontic life history for a species of Enteromorpha (Link). The population then had to be classified to species, and here major difficulties were encountered. Chapman's (1956) key to the New Zealand species was used.

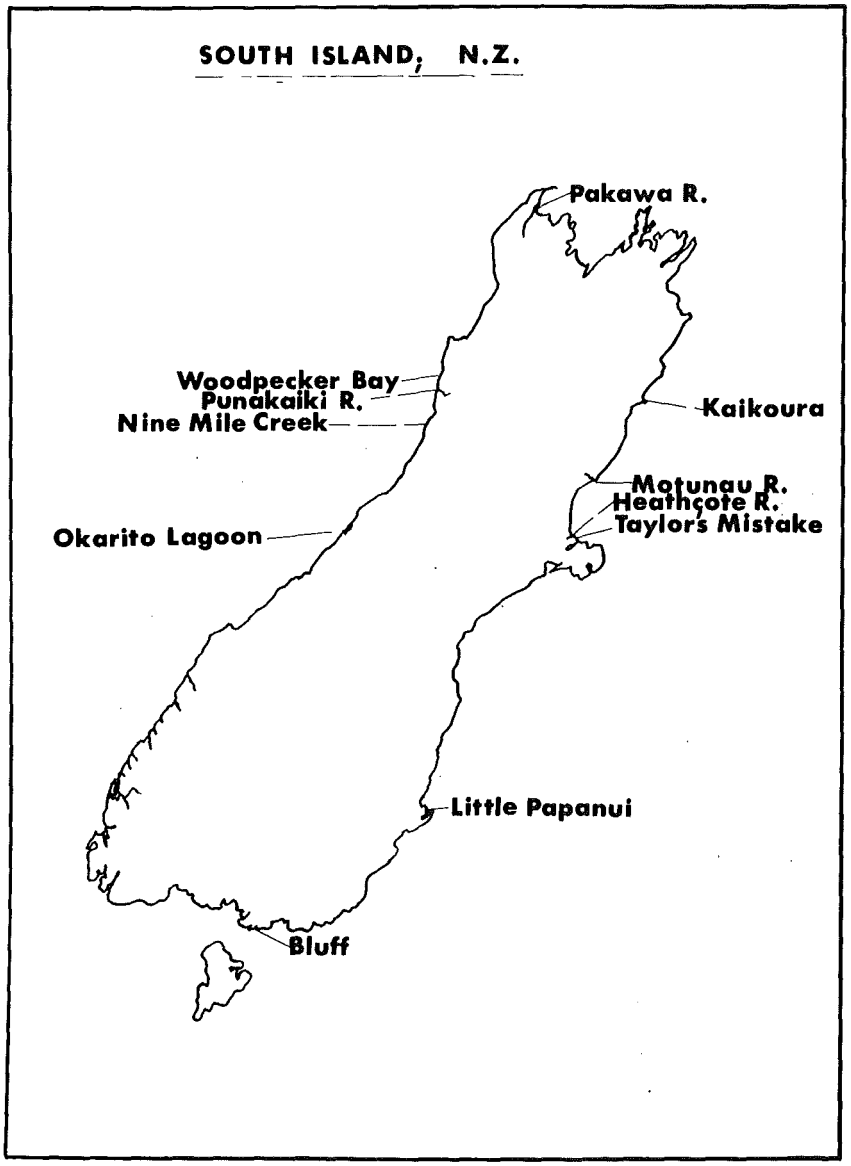


FIGURE 1 - The places in the South Island, New Zealand at which collections of Enteromorpha were made for this thesis.

Several characters on which this was based were found to be so ambiguous that the population could be fitted into any of several species. Besides, a satisfactory classification could not be made by a systematic attempt to fit the characters of the Motunau population to any one of the 47 individual type descriptions recognised by Chapman. A similar situation was found in nearly every Enteromorpha population examined by the writer.

A detailed account of the attempts to classify any one of these populations would be undesirable in a work of this nature. However, for each population, every character appeared to vary to some extent. While a certain amount of variation in some characters would, of course, be expected, the lack of a single stable character created a difficult taxonomic situation. The diagnostic characters included gross morphology, chloroplast morphology, number of pyrenoids, width of the mucilage envelope, cell size and arrangement, and thallus colour.

It was concluded that in most Enteromorpha populations each character had a natural range of variation. (This would have to be accurately recorded before any satisfactory method of separating species could be devised.)

A series of observations and experiments was begun in January 1965 with a view to determining the following:-

(1) The variation in gross* morphology of one population at any one time of the season.

---

* The term 'gross' is used here to mean the general form of the plant as distinct from chloroplast morphology.

(2) The range of variation of the following features in a statistically significant section of an Enteromorpha population at any one time of the season:-

A. chloroplast morphology

B. chloroplast orientation

C. chloroplast colour

D. presence (and number) or absence of pyrenoids

E. cell size

F. width of the mucilage envelope

G. degree of convolution and its position on the thallus.

The Variation in Gross Morphology of a Population at any one Time During the Growing Season.

Most references in the literature simply record the fact that Enteromorpha is a variable genus. Few are made to particular taxonomic criteria and their individual ranges of variation.

Chapman (1956) described Enteromorpha as 'A notoriously difficult Genus. I believe that there are only five, possibly six Linnaean species, and the remaining so-called species are forms and varieties of these': Womersley (1956) noted that few genera were as troublesome as Enteromorpha. According to Bliding (1963) 'It is well known that in the Genus Enteromorpha many species vary exceedingly in regard to size, colour, ramification and other external morphological qualities'.

Gross morphology is believed by some to be of little taxonomic significance (Smith, 1950; Bliding, 1963). Other workers have commented on the variation in gross morphology within a single population,

without discussing the taxonomic implications. Arasaki and Shihira (1959) described the difference in Ulva (Enteromorpha) Linza at the two extremes of the littoral zone. Weeks (1961) noted the same effect in Enteromorpha intestinalis without commenting further.

In spite of the limitations of gross morphology as a means of separating individual species several workers, including Taylor (1937, 1960) and Chapman (1956) have employed this character extensively, but with reservations imposed by its variability.

Taylor (1960) qualifies by noting the extreme likelihood of its variation. Under these circumstances, he adds, 'the relatively stable structural characters of cell shape, size, and arrangement will usually give the necessary clues to enable identifications to be made'. Womersley (1956) utilized these "relatively stable structural characters" in delimiting the Southern Australian Enteromorphas. However, in view of Bliding's earlier work, he noted that their validity was doubtful.

Morphological variation has also been related to habitat. Dangeard (1957) noted the correspondence between stipe branching and sandy substrata, in normally unbranched Enteromorpha flabellata. This was regarded as a habitat variant within the species. Similarly Scagel (1963) inclined to the view that the alga known as Collinsiella tuberculata Setchell and Gardner was simply an extreme habitat form of Enteromorpha intestinalis. According to this point of view, the concept of the species was wide enough to accommodate extreme habitat variants.

On the other hand, Chapman (1956) created taxa (ecads) solely to include habitat variants e.g. Enteromorpha nana var. minima ecad rivularis.

On other occasions he simply noted the likelihood of certain forms being environmentally induced variants e.g. E. bulbosa f cornucopiae form. nov. 'This form may represent a seasonal condition, but the information available at present is not sufficient to justify this assumption'.

Bliding (1963 and earlier papers) regards reproductive barriers as the only reliable means of distinguishing species. By growing known reproductively distinct populations in artificial cultures it is possible to study the relatively stable morphological and anatomical criteria for each species.

From the above it may be concluded that the relative importance accorded to gross morphology varies with the worker. It is generally agreed that this criterion is variable. However, it is not generally agreed that the determination of reproductive barriers provides a practical answer to the problem of distinguishing readily individual species.

Reliability of Gross Morphology as a Taxonomic Criterion.

In the present investigation initial attempts to classify several populations met with no success. Gross morphology was one of those characters referred to above which could not be used satisfactorily to identify different species. Therefore particular attention was given to variation of this character throughout the series of investigations.

An examination of the literature revealed that, in several systematic accounts of the group, the morphological characteristics recorded for various species (Taylor 1937, 1960) and a subspecies (Chapman, 1956) were indistinct. Morphology has become critical in these cases because there are no other definitive characters.

The following may serve as an example. Taylor (1960) made no great morphological distinction between Enteromorpha intestinalis (Linnaeus) Link and E. compressa (Linnaeus) Greville.

Enteromorpha intestinalis (Linnaeus Link)

'Solitary or gregarious subintestiniform, 1 - 20 dm. or even more in length. 1 mm - 10 cm wide, membranous; frond tapering below, above the stalk elongate - attenuate, tubular, cylindrical, clavate or generally inflated and bulbate; simple, or rarely and sparingly branched from the very base, or proliferous; cells not arranged in any order in the inflated portion - in surface view 9 - 15 μ diameter - the whole membrane 20 - 40 μ thick.'

Enteromorpha compressa (Linnaeus) Greville

'Plants generally gregarious, to 3 dm tall, tubular more or less compressed or collapsed; above expanded 2 - 20 mm wide; below long, tapering and characteristically with several branches from the gradually contracted stalklike base which are similar to the principal blade, cells in the adult plants irregularly placed, 10 - 15 μ diameter, - the whole membrane 13 - 20 μ thick.'

The difference between these two species is largely one of size. However, those E. intestinalis plants within the region of overlap (a thallus width of 2 - 20mm) should still be separable from E. compressa by the possession of an inflated and bullate thallus.

According to Bliding (1963) this feature may develop in E. intestinalis as a result of a rise in water temperature. In addition, the figures of Enteromorpha compressa var compressa (Fig. 82a., P.133) and E.compressa var usneoides (Fig. 85c., P.137) show convolution similar to that in

E. intestinalis var intestinalis (Fig. 87a. - d., P.140, Bliding, 1963). However, neither Taylor nor Bliding mention any convolution for E. compressa.

The small E. intestinalis plants are therefore not separable from E. compressa by means of the gross morphological characters supplied by Taylor (1960). Bliding separated the two by failure of their gametes to copulate (reproductive isolation). This method was employed by the same worker in a more extensive survey of N. American species (quoted Kylin, 1949). However, Taylor (1960) concludes that 'it is not possible to apply the studies of Bliding and others (Kylin) on southern races, ... to these tropical species at this time'.

During the present investigation considerable difficulty was experienced in obtaining release of reproductive bodies. This writer has, for practical reasons, reached the same conclusion as Taylor (1960) regarding the application of Bliding's studies to taxonomy.

As a result of the somewhat inadequate morphological records in the literature, many of the local populations examined in the early stages of this study appeared to contain individuals of several species. Bearing the nature of these records in mind, it was postulated that every Enteromorpha population contained a range of variation in almost every feature. If the range of each was known, it might be possible to make morphology more meaningful for taxonomic purposes. Recognition of general variation in diagnostic characters is one of the basic premises of modern taxonomy of higher plants. It may well be that earlier workers in the taxonomy of algae attempted to take this into consideration.

THE VARIATION IN GROSS MORPHOLOGY OF ONE POPULATION AT ANY ONE TIME OF THE SEASON

Method.

At every locality visited (Figure 1) a large number of plants growing in close proximity to one another, were collected. Full field notes were kept particularly pertaining to any unusual features of the environment. The individual plants were separated and mounted on herbarium sheets in the laboratory.

From the outset of this study, it was found that extreme forms of habit occurred in many populations. These were included only when (1) their microscopic characters corresponded with those of the population generally and (2) a range of intermediate habit between them and the rest of the population could be found. In most cases these conditions were easily fulfilled.

Result.

It soon became very clear that every Enteromorpha population examined had a range of gross morphology. A population collected from the midlittoral zone Woodpecker Bay, West Coast, South Island, on 13.5.65 may be taken as an example.

The microscopic characters of this population were as follows:- average diameter of cells in the median region, 10μ ; chloroplast homogeneous, hood shaped; grass green in colour. The range in gross morphology is shown in Figure 2.

To the best of the writer's knowledge there was nothing unusual in the environment of this population. It grew in a shallow pool in the

rocks, which protected it from any direct wave action and from long periods of direct illumination. The grass green colouration was probably due to the filtering effect of the permanent shallow layer of water.

In view of these and numerous similar results, it can only be concluded that a range of gross morphology is a normal feature of any Enteromorpha population. It appears that not enough consideration is given to this in the two main taxonomic works on the Genus; e.g. Chapman (1956) refers to the gross morphology of Enteromorpha intestinalis (L) Grev. 'frond as simple, very occasionally branched', E. intestinalis f. tubulosa Kutz 'simple or with hair like branchlets'; e.g. Bliding (1963) refers to E. intestinalis var asexualis f cornucopiae Lyngbye 'broad, sometimes cornet-shaped, generally wrinkled thallus with quite a short stipe which is often proliferous'. The figure to which this comment refers shows a range of form (Fig. 91b., P.146). In addition, Bliding (1933, 1944, 1963) has recorded a range of gross morphology in E. clathrata "Between Typus I, II and III occur transition forms" 1963, P.109.

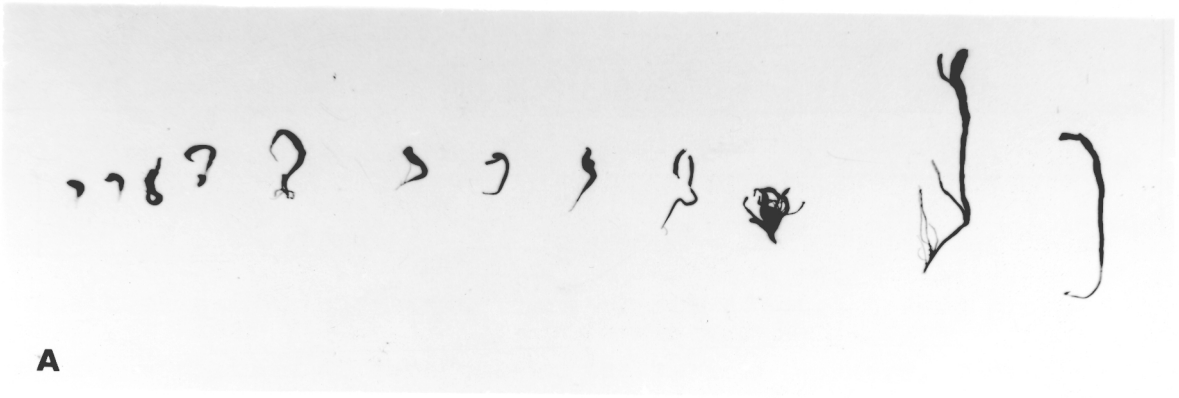
While Bliding appears to be aware of the situation, it is not at all clear whether Chapman is. Neither have recorded a range of gross morphology as it would be presented in a higher plant taxonomic treatise. Until algal taxonomists conform with the principle of accurately describing or delimiting any range of variation they find, little progress will be made with the taxonomic impasse in Enteromorpha.

FIGURE 2 (opposite)

The range in gross morphology of a population collected from the midlittoral zone, Woodpecker Bay, West Coast, South Island.

The general range is shown in A. More detailed variation of those plants from the small end of the size scale is shown in B, from the median region of the scale in C and large end of the scale in D.

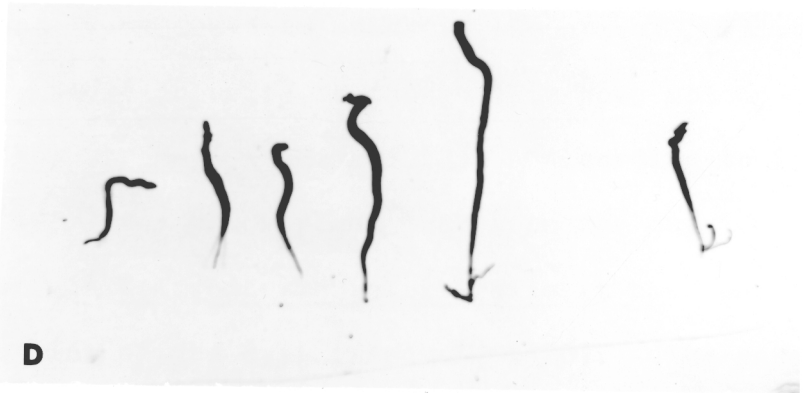
These subdivisions are purely arbitrary, their sole purpose being to illustrate the range of variation in greater detail.



A

B

C



D

FIGURE 2

THE RANGE OF VARIATION IN SOME TAXONOMIC CHARACTERS IN A STATISTICALLY SIGNIFICANT SECTION OF AN ENTEROMORPHA POPULATION: A CRITICAL APPRAISAL

Introduction and Literature.

It has been established that the approach adopted toward gross morphological variation differed among the various workers. Chloroplast morphology, for example, is also described in various terms.

Chloroplast morphology. Fritsch (1935) defines the type of chloroplast in the Genus as 'a single parietal chloroplast with deeply incised or lobed margins'. According to Smith (1956) in the Ulvaceae is found a single laminate to cup-shaped chloroplast on the outer face of the cell. Chapman (1956) in his treatise on the New Zealand species of Enteromorpha distinguishes several types including sac-like, homogeneous and granular chloroplasts. According to Taylor (1960) the members of the genus had a single lateral plate-like chloroplast covering the outer or lateral face of the cell. Other workers do not include this character in their definition of the Genus (e.g. Bliding, 1963).

The latter does, however, include chloroplast shape, orientation and additional details such as lobing in some definitions of individual species.

The chloroplast is believed to occur in the apical portion of the cell, and thereby exhibit polarity, although this does not appear to apply in Ulva and Monostroma (Chapman, 1962). According to Fritsch (1935) and Smith (1938) the chloroplast occurs on the outside wall of the cell. However, in Enteromorpha intestinalis it has been shown to occur on the lower side of the cell (Henckel, 1926). The report for

this species is therefore contrary to the definitions of Smith (1955) and Taylor (1960).

Pyrenoids. The number of pyrenoids per cell varies from 1 - many (Bliding, 1944). These are of great importance as a distinguishing character (Bliding, 1933, 1963; Waern, 1952; Dangeard, many papers in 'Le Botaniste'). Fritsch (1935) and Smith (1955) stated that Enteromorpha had only one pyrenoid per cell.

Cell size. Cell size and arrangement has generally been used as a character definitive of individual species or species groups (Taylor, 1937, 1950; Chapman, 1956; Womersley, 1956; Bliding, 1963; Scagel, 1963; Dangeard, 1958a.). It has been shown in case of E. Linza, growing in eutrophic water, that the cell size is different from that in normal sea water (Bliding, 1963).

The two remaining characters - amount of convolution and its position on the thallus, and width of the mucilage envelope, are used in the same way as cell size, by the authors noted in the preceeding paragraph.

From the above it may be concluded that amongst those working on Enteromorpha, few individual taxonomic characters are used uniformly of which chloroplast morphology and orientation are examples.

Methods.

From the outset it was decided that this series of investigations should embrace as many Enteromorpha populations from as widely separated geographical areas as possible. It was believed that only by this means could significant conclusions be drawn for the Genus in the South Island as a whole. Therefore the population sample for the present experiment was collected from the Bluff region.

Whilst it was necessary to select a population at random from the region, an element of human bias was unavoidable. Only a one day visit could be arranged to Bluff, and on this day high tide occurred at 2 p.m. As a result, only rocks in the upper littoral zone were accessible for collecting. Enteromorpha will not establish on mudflat (personal observation). Ultimately a population adjacent to the Ocean Beach Freezing Co. was studied.

Two stones, about 20' apart, clothed in a young Enteromorpha population were chosen. With the aid of a scalpel, a large number of plants were removed from each rock. The two samples were marked 'A' and 'B', placed in quart preserving jars of sea water, and transferred to the laboratory in Christchurch the following day.

Each plant was then separated from any to which it happened to be joined by a common holdfast. Twenty specimens were then removed from the jars quite at random, for the herbarium.

The range of gross morphological variation is illustrated in Figures 3 - 6. It varied from:- unbranched plants with convolute and non-convolute regions of equal length, Figure 3; plants with sparsely branched convolute regions, Figure 4; to plants with sparsely or

profusely branched non-convolute regions, Figures 5 and 6 respectively.

Table 1 sets out the variations of the remaining characters recorded. For this purpose 50 plants were used, 25 each from samples 'A' and 'B', taken at random. An additional 10 minute plants, all that could be located in the sample, were recorded. It was hoped that these would indicate any ontogenetic changes occurring in the selected characters.

Explanation of some of these is in order. Thallus length and width: Every plant was first examined microscopically to ensure that it possessed a holdfast and was thereby intact. Thallus length and width was then recorded in cm and mm respectively. The width was always taken as the widest point, but most plants possessed an average slightly less than this value. The object of these measurements was to detect any correlation of thallus size with the other characters. The dimensions of the 50 plants are shown in Table 2.

For the purpose of recording the remaining characters, each plant was divided into thirds, and a section of thallus removed from each with the aid of a scalpel. (That section of the thallus in which the cells had contributed rhizoids to the holdfast was excluded from this consideration. This region is not normally utilized in taxonomy). The three sections were designated, from top to bottom, distal, median and basal. Each was taken as representative of distal, median and basal regions of the thallus.

Thallus form: Each section was classified as convolute or non-convolute. The results for basal, median and distal regions are shown in Tables 3,4, and 5 respectively.

TABLE 1 - Summary of Variations in Taxonomic Features, and Methods of Recording.

<table>
<tr><td>Taxonomic Character</td><td colspan="5">Recording technique</td></tr>
<tr><td></td><td colspan="5">Recorded once for each plant</td></tr>
<tr><td>Thallus length cm and width mm</td><td colspan="5">Width of the widest part only recorded. Most plants were less than the recorded value over most of their length.</td></tr>
<tr><td></td><td colspan="5">Recorded for the basal, median and distal regions of each plant.</td></tr>
<tr><td>Thallus Form</td><td colspan="5">Convolute or non-convolute</td></tr>
<tr><td>Cell Diameter</td><td>The longest diameter was measured for 10 cells</td><td colspan="4">The average of these 10 measurements was calculated</td></tr>
<tr><td>Chloroplast morphology</td><td>The range was reduced to 16 general types</td><td colspan="4">Each type was assigned a number (1 - 16) and drawn to scale.</td></tr>
<tr><td rowspan="3">Chloroplast orientation and pattern</td><td colspan="5">Chloroplasts not orientated - no pattern</td></tr>
<tr><td colspan="5">Chloroplasts oriented - pattern orientated towards:-</td></tr>
<tr><td colspan="5"><table><tr><td>Top</td><td>Side</td><td>Top Side</td><td>Side & Bottom</td><td>Bottom</td></tr><tr><td colspan="5">of thallus</td></tr></table></td></tr>
<tr><td>Chloroplast Colour</td><td>Varies under microscope according to light intensity and magnification</td><td colspan="4">Colour was recorded under x600 magnification</td></tr>
<tr><td>Pyrenoids</td><td colspan="5">Present or absent from the majority of cells examined</td></tr>
<tr><td>Mucilage envelope</td><td>Only the mucilage on the outside of the monostromatic cell layer was recorded</td><td colspan="4">width in μ</td></tr>
</table>

TABLE 2 - Bluff Population: Thallus size and cell diameter.

Plant No.	Plant Size		Cell Diameter			
	Thallus length cm	Thallus width mm	Average for Basal Region	Average for Median Region	Average for Distal Region	Average for the whole plant
1	15	1.5	4.75	5.13	7.68	5.85
2	7	1.5	4.25	5.38	7.35	5.56
3	10	1.5	4.13	5.00	5.00	4.70
4	3.5	1	4.63	5.88	6.13	5.54
5	5	1.5	4.38	6.63	6.13	5.71
6	7	1.5	4.25	6.00	5.50	5.25
7	6.5	1.5	4.50	5.13	7.58	5.73
8	3	2	4.25	4.25	5.13	4.54
9	3.5	3	4.00	5.25	4.75	4.66
10	1.5	1.25	4.25	4.25	4.63	4.37
11	3	1.5	4.38	5.13	5.13	4.88
12	3.5	2	4.25	5.13	4.13	4.50
13	5	1	4.88	4.63	5.00	4.83
14	3.5	.75	4.63	5.38	6.25	5.48
15	4.5	1.25	4.75	5.63	7.35	5.91
16	14	2.5	6.00	5.13		3.71
17	4	1	4.50	5.25	6.43	5.39
18	1.5	.5	4.88	5.13	4.88	4.96
19	11	1	4.88	5.38	6.00	5.42
20	9.5	1.5	4.88	6.43	4.88	5.39
21	9.5	1.5	5.38	5.00	4.75	5.04
22	6.5	1	4.63	5.00	5.25	4.96
23	2.5	2	4.63	4.88		4.75
24	3	3	4.63	4.25	5.25	4.71
25	7	1.5	4.60	6.00	8.08	6.22

TABLE 2 (CONT.)

Plant No.	Plant Size		Cell Diameter			
	Thallus length	Thallus width	Average for Basal	Average for Median	Average for Distal	Average for the whole plant
26	2	2	6.00	5.80	4.88	5.56
27	2.5	2	4.50	5.75	5.38	5.21
28	2	1.5	5.00	5.25	5.88	5.37
29	4.5	1	4.88	4.88	6.08	5.28
30	2.5	1.25	4.75	4.88	7.40	5.67
31	5.6	1	4.75	5.50	5.75	5.33
32	8	1.25	4.88	6.25	6.75	5.96
33	6.5	1	4.25	4.88	4.75	4.62
34	4.5	2	5.75	5.00	5.50	5.41
35	4.5	1	5.00	6.00	5.25	5.41
36	3	1	4.75	5.38	5.38	5.17
37	4	1	5.50	5.50	4.63	5.21
38	4	1	5.13	4.88	5.63	5.21
39	3.8	1	5.13	6.38	5.88	5.79
40	4.5	1	4.88	5.25	4.63	4.85
41	3	.25	4.63	4.38	4.50	4.50
42	2.5	.75	5.25	6.10	6.83	6.06
43	7	1	4.88	5.13	5.45	5.15
44	2.75	2	5.13	4.88	6.75	5.58
45	5	1	4.50	4.75	5.50	4.91
46	5.5	1	4.75	5.75	6.13	5.54
47	3	1	4.37	5.60	6.28	5.45
48	6.5	1.25	4.63	4.43	5.65	4.90
49	6	3	4.63	4.50	5.58	4.90
50	8	1.5	5.68	4.85	4.93	6.48

TABLE 3 - Bluff Enteromorpha Population Sample: Basal Region: Variation of Taxonomic Characters.

Plant No.	Thallus Form		Chloroplast Morphology			Chloroplast Orientation							Chloroplast Colour		Pyrenoid		Mucilage Envelope		
	Convolute	Non-Convolute	Chloroplast Type No.	Chloroplast Type No.	Modified	Orientated	Not Orientated	Top	Top and Side	Side	Side and Bottom	Bottom	Dark Green	Light Green	Present	Absent	Absent	Present	Width
1		X	8				X						X			X	X		
2		X	4		X	X				X			X			X	X		
3		X	4		X	X		X					X			X	X		
4		X	4		X		X							X		X	X		
5		X	6	16	X		X						X			X	X		
6		X	6				X						X			X	X		
7		X	5				X						X			X	X		
8		X	4				X							X		X	X		
9		X	9		X	X				X			X			X	X		
10		X	6	9	X	X			X				X			X	X		
11		X	4				X						X			X	X		
12		X	6				X						X			X	X		
13		X	7				X						X			X	X		
14		X	6		X		X						X			X	X		
15		X	3				X						X			X	X		
16		X	9		X		X							X		X	X		
17		X	6				X						X			X	X		
18		X	6				X						X			X	X		
19		X	7		X	X					X		X			X	X		
20		X	4				X						X			X	X		
21		X	4	7			X						X			X	X		
22		X	7				X						X			X	X		
23		X	7				X							X		X	X		
24		X	7				X						X			X	X		
25		X	3			X				X			X			X	X		

TABLE 3 (CONT.)

Plant No.	Thallus Form		Chloroplast Morphology			Chloroplast Orientation							Chloroplast Colour		Pyrenoid		Mucilage Envelope		
	Convolute	Non-Convolute	Chloroplast Type No.	Chloroplast Type No.	Modified	Orientated	Not Orientated	Top	Top and Side	Side	Side and Bottom	Bottom	Dark Green	Light Green	Present	Absent	Absent	Present	Width
26		X	7			X						X	X			X	X		
27		X	6		X		X						X			X	X		
28		X	6	14		X					X		X			X	X		
29		X	4		X	X					X		X			X	X		
30		X	6				X						X			X	X		
31		X	7				X							X		X	X		
32		X	4		X	X					X			X		X	X		
33		X	6		X		X						X			X		X	7.5
34		X	6		X		X						X			X		X	10
35		X	5				X						X			X	X		
36		X	6				X						X			X	X		
37		X	6				X						X			X	X		
38		X	6				X						X			X	X		
39		X	6				X						X			X		X	7.5
40		X	14				X						X			X		X	7.5
41		X	14				X						X			X		X	9.25
42		X	12				X						X			X	X		
43		X	13				X						X			X		X	8.25
44		X	12				X						X			X		X	4.25
45		X	12				X						X			X		X	9.25
46		X	4				X						X			X		X	3.75
47		X	6				X						X			X	X		
48		X	6		X		X						X			X		X	7.5
49		X	6				X						X			X		X	10
50		X	6				X						X			X		X	8.25

TABLE 4 - Bluff Enteromorpha Population Sample: Median Region: Variation of Taxonomic Characters.

Plant No.	Thallus Form		Chloroplast Morphology			Chloroplast Orientation							Chloroplast Colour		Pyrenoid		Mucilage Envelope		
	Convolute	Non-Convolute	Chloroplast Type No.	Chloroplast Type No.	Modified	Orientated	Not Orientated	Top	Top and Side	Side	Side and Bottom	Bottom	Dark Green	Light Green	Present	Absent	Absent	Present	Width
1		X	4	5	X		X							X		X	X		
2	X		4	8	X		X							X		X	X		
3	X		1				X							X		X	X		
4	X		8			X						X		X		X	X		
5		X	4	5			X							X		X	X		
6	X		5				X							X		X	X		
7		X	4				X							X		X	X		
8	X		4				X							X		X	X		
9	X		6		X		X							X		X	X		
10		X	4				X							X		X	X		
11	X		3			X					X			X		X	X		
12	X		3		X		X							X		X	X		
13			1	4	X	X					X			X		X	X		
14		X	4			X		X						X		X	X		
15		X	5			X						X		X		X	X		
16	X		4	9	X	X						X		X		X	X		
17	X		4	9	X		X							X		X	X		
18		X	6	4	X		X							X		X	X		
19	X		7			X				X				X	X		X		
20	X		4				X						X			X	X		
21	X		7			X						X		X		X	X		
22	X		5				X							X		X	X		
23	X		4				X						X			X	X		
24	X		7				X							X		X	X		
25	X		4	7	X	X						X		X		X	X		

TABLE 4 (CONT.)

Plant No.	Thallus Form		Chloroplast Morphology			Chloroplast Orientation							Chloroplast Colour		Pyrenoid		Mucilage Envelope		
	Convolute	Non-Convolute	Chloroplast Type No.	Chloroplast Type No.	Modified	Orientated	Not Orientated	Top	Top and Side	Side	Side and Bottom	Bottom	Dark Green	Light Green	Present	Absent	Absent	Present	Width
26	X		4	9			X							X		X	X		
27		X	6	3			X							X		X	X		
28		X	4	5			X							X		X	X		
29		X	4		X	X						X		X		X	X		
30	X		7			X						X		X		X	X		
31	X		7				X							X		X	X		
32	X		12				X						X			X	X		
33	X		4	5		X						X		X		X	X		
34	X		9				X							X		X	X		
35		X	12				X							X		X	X		
36		X	4				X							X		X	X		
37	X		11				X							X		X	X		
38		X	4				X							X		X	X		
39		X	4	7			X						X		X	X	X		
40	X		4				X							X		X	X		
41		X	6	9	X		X							X		X	X		
42		X	4				X						X			X	X		
43		X	5				X							X		X	X		
44		X	4	9	X		X							X		X	X		
45		X	4				X							X		X	X		
46	X		12				X							X		X		X	.75
47		X	12				X						X			X	X		
48		X	9		X		X							X		X		X	2
49	X		9		X		X							X		X	X		
50	X		9		X		X							X		X		X	2

TABLE 5 - Bluff Enteromorpha Population Sample: Distal Region Variation of Taxonomic Characters.

Plant No.	Thallus Form		Chloroplast Morphology			Chloroplast Orientation							Chloroplast Colour		Pyrenoid		Mucilage Envelope		
	Convolute	Non-Convolute	Chloroplast Type No.	Chloroplast Type No.	Modified	Orientated	Not Orientated	Top	Top and Side	Side	Side and Bottom	Bottom	Dark Green	Light Green	Present	Absent	Absent	Present	Width
1	X		6				X						X			X	X		
2	X		1	3	X		X						X			X	X		
3	X		1		X		X						X			X	X		
4	X		4		X		X						X			X	X		
5		X	3		X		X						X			X	X		
6	X		4	5		X						X	X			X	X		
7		X	6				X						X			X	X		
8	X		3		X		X						X			X	X		
9	X		4				X							X		X	X		
10	X		4				X						X			X	X		
11	X		5		X	X		X					X			X	X		
12	X		7			X				X			X			X	X		
13		X	7		X	X			X				X			X	X		
14	X		4				X						X			X	X		
15		X	1	4			X						X	X		X	X		
16	X															X	X		
17	X		9	1			X						X	X		X	X		
18		X	4				X						X			X	X		
19	X		7	4			X						X			X	X		
20		X	6				X						X			X	X		
21	X		4	1			X						X			X	X		
22	X		5	1			X						X			X	X		
23																X	X		
24	X		5				X						X			X	X		
25	X		4				X						X			X	X		

TABLE 5 (CONT.)

Plant No.	Thallus Form		Chloroplast Morphology			Chloroplast Orientation							Chloroplast Colour		Pyrenoid		Mucilage Envelope		
	Convolute	Non-Convolute	Chloroplast Type No.	Chloroplast Type No.	Modified	Orientated	Not Orientated	Top	Top and Side	Side	Side and Bottom	Bottom	Dark Green	Light Green	Present	Absent	Absent	Present	Width
26	X		4	9		X		X					X			X	X		
27		X	7			X		X					X			X	X		
28	X		4	9		X						X	X			X	X		
29		X	12				X						X			X	X		
30	X		12		X		X						X			X	X		
31	X		4				X						X			X	X		
32	X		12		X		X							X		X	X		
33	X		12				X						X			X	X		
34	X		12				X						X			X	X		
35		X	12				X						X			X	X		
36		X	12				X						X			X	X		
37	X		4				X						X			X	X		
38	X		12			X			X				X			X	X		
39		X	4				X						X			X	X		
40	X		11				X						X			X	X		
41		X	12				X						X			X	X		
42		X	4		X		X							X		X	X		
43		X	5				X						X			X	X		
44		X	4	9	X		X						X			X	X		
45		X	4				X						X			X		X	.75
46	X		12		X		X						X			X	X		
47		X	12				X						X			X	X		
48		X	9		X		X						X			X	X		
49	X		9		X		X						X			X	X		
50	X		9		X		X						X			X		X	2

Cell Diameter: In each section the longest diameter of 10 cells was measured. Randomisation of the sample was effected in the following way. After each individual measurement the micrometer eyepiece was rotated a short distance in the microscope tube. A cell in the centre of the field, on which the scale markings rested closely against the farthest outside walls was measured. The results were averaged for each region, and for the three regions of each plant (Table 2).

Chloroplast Morphology: A preliminary survey of chloroplasts from several plants revealed a great diversity of form. Extensive experimentation with various photographic techniques showed that photographs were invaluable for recording the general form of plants and cells, but did not bring out fine detail. A series of line diagrams was therefore used for recording chloroplast morphology.

The range of variation was reduced to as few general forms as possible. Ultimately 16 representative forms were distinguished, from the 50 adult and 10 juvenile plants. The 'type diagram' in each case was drawn from an actual specimen. Each was assigned numbers from 1 - 16, and the appropriate number for each of the three regions recorded against the plant number. Tables 3, 4, and 5 show the results for the basal, median, and distal regions respectively.

In practice it was found that some plants had chloroplasts of two recognised forms within the area of a low power microscope field. Both were recorded in these cases. More frequently it was found that a chloroplast did not agree closely with any of the 16 recognised forms. These were recorded under the number of the chloroplast they resembled most closely, with an additional positive notation in the 'modified' column.

Chloroplast orientation: As each plant was cut into the three sections representing basal, median and distal regions, care was taken that they remained correctly orientated with respect to the top of the thallus. Whilst it was possible to recognise a common chloroplast orientation for cells in some sections, many more did not conform to any pattern. Therefore the following categories were recognised with respect to chloroplast orientation in each section.

Either (a) chloroplast unorientated

or (b) chloroplast orientated -

generally towards the

(1) top of the thallus

or (2) top and side of the thallus

or (3) side of the thallus

or (4) side and bottom of the thallus

or (5) bottom of the thallus.

The detailed record for the three regions of each plant appear in Tables 3, 4, and 5.

Chloroplast colour: It was found that the chloroplasts varied slightly in colour with varying magnification and or light intensity. In order to standardise the recording technique, as far as possible, recordings were made under high power. This necessitated using the Riostat at almost full intensity, thus 'standardising' the light source. The colour of the majority of cells in each section was that recorded in Tables 3, 4, and 5. Where there were some minor colour variations, these were noted on the appropriate figures where necessary.

Pyrenoids: A preliminary survey of the occurrence of pyrenoids in this population revealed that they were absent or not clearly visible in most cells. Iodine solutions of various formulae have been extensively employed by phycologists and botanists in general, as a test for starch (e.g. Bliding, 1963). However, the use of iodine was ruled out in the present investigation, on the following grounds.

In order for the stain to react with the starch sheath about each pyrenoid, it must diffuse into the cell. A concentration sufficient for a positive starch test causes a colour cast over the entire cell. Under these circumstances, in certain planes it is difficult to distinguish lobed extensions of a chloroplast from discrete circular bodies. It is therefore difficult to distinguish lobes of the chloroplast from stained pyrenoids. Both have a heavy dark boundary. That of the chloroplast lobe is caused by the high iodine content in the lumen and plastid, while the pyrenoid has a dark boundary due to a positive starch iodine reaction.

Many cells possessed a body which could have been a pyrenoid. However, the writer could only positively identify a few as pyrenoids, on the basis of personal experience with other populations.

The presence or absence of pyrenoids was recorded for the basal, median, and distal regions on Tables 3, 4, and 5 respectively.

Mucilage Envelope: This character is normally expressed as the total width of envelope i.e. from the centre to the outside of the thallus including the cells. This necessitates cutting transverse sections for every measurement. However, time would not permit such a lengthy procedure for the present investigation. Instead, the width of mucilage envelope outside the cell layer was measured. This was readily visible

without sectioning. On many occasions there was no measureable thickness of mucilage in this position. For each of the three regions, the presence (and thickness in μ) or absence of a mucilage envelope was recorded.

It has been shown that the cell layer may occasionally shift its position with respect to the outside of the mucilage envelope (Bliding, 1963). It was assumed, for the purposes of the present investigation, that this had not occurred. The results must therefore be considered with this reservation.

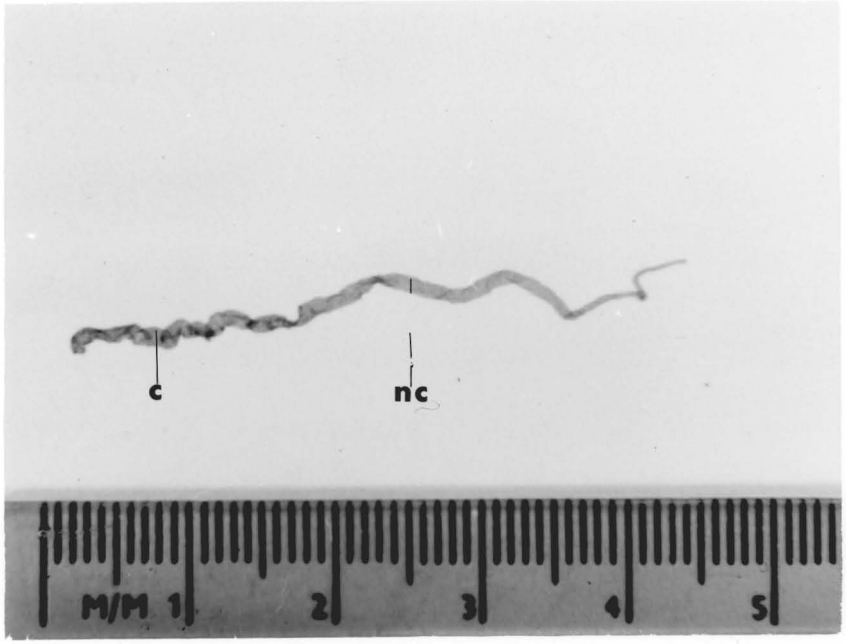


FIGURE 3 - An unbranched plant from Bluff with an equal length of convolute (c) and non-convolute (nc) thallus.

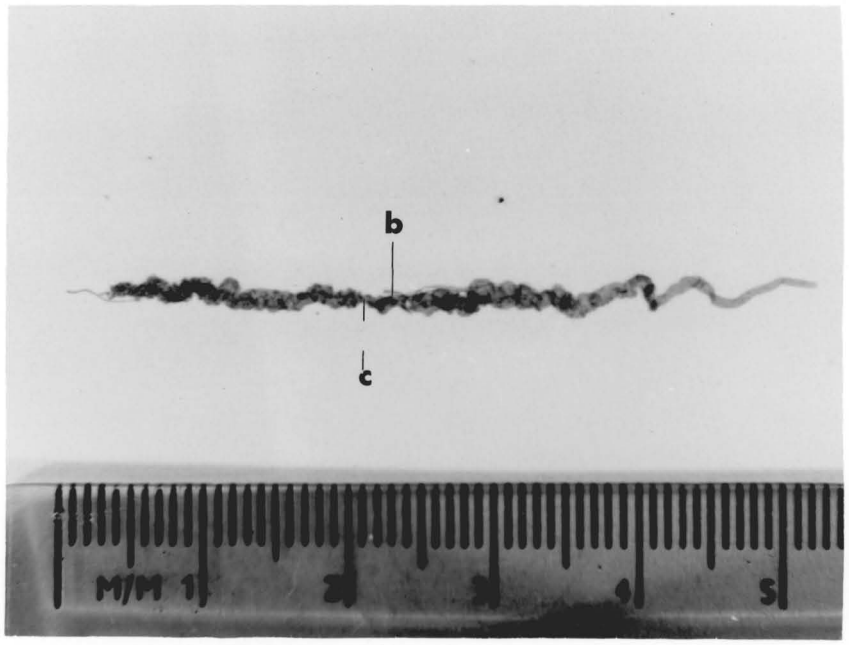


FIGURE 4 - A branched plant with an extensive convolute region (c) bearing a few fine branches (b).

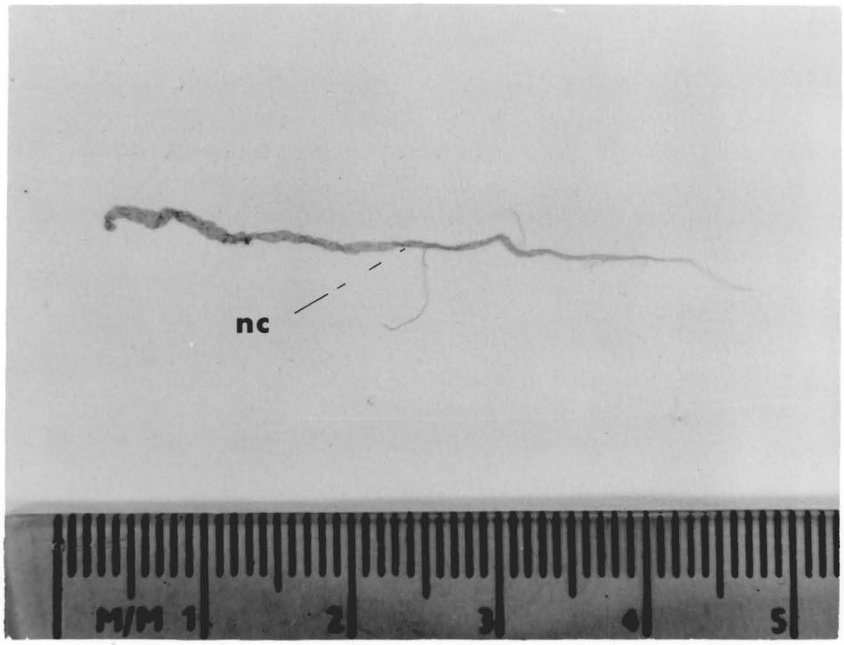


FIGURE 5 - A plant with a sparsely branched non-convolute region (nc).

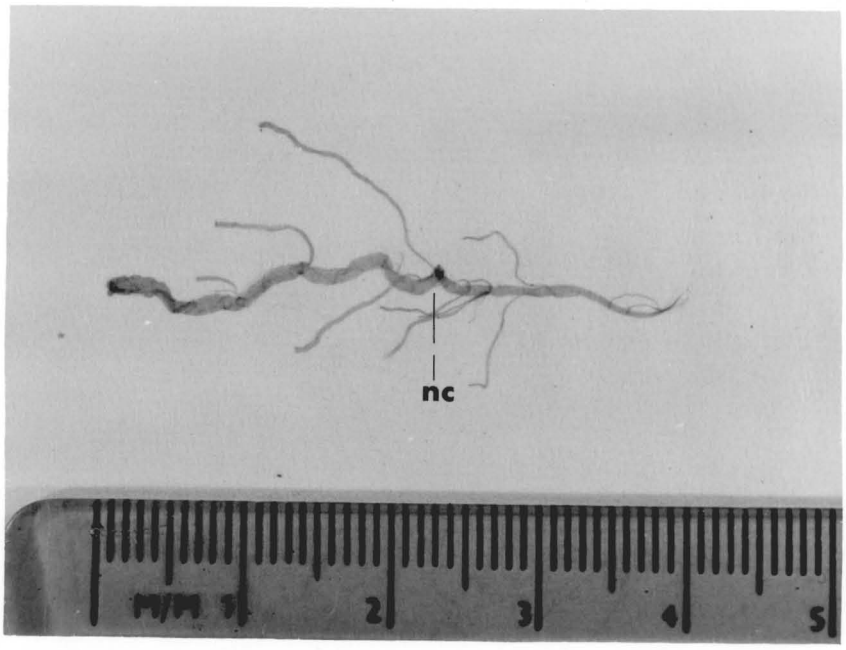


FIGURE 6 - A plant collected from Bluff with a number of branches arising from the non-convolute (nc) region of the thallus.

Results.

In the following section, the results and conclusions for each taxonomic character are discussed in turn. Some were not investigated to determine their worth as taxonomic characters in themselves, but rather to correlate with others. These paragraphs, for example thallus size, are therefore very brief.

Thallus size. The 50 adult plants examined varied from 1.5 cm to 15 cm long and from .25 mm to 3 mm wide. The average length was 5.22 cm, and average width 1.41 mm.

The purpose of recording this character was to detect any correlation of thallus size with variation in the following characters.

Thallus form: Table 6 summarises the results for the 3 regions. 54% of the median regions and 62% of the distal regions examined were classed as convolute. 12% of the sample possessed convolute distal regions only, and 4% convolute median regions alone. 50% of the population possessed both convolute median and distal regions. On the basis of this evidence there was approximately an equal chance of any plant in the population possessing this taxonomic character.

However, should convolution have correlated well with another easily recognisable taxonomic character, it might have been possible to utilize it as an associate character, perhaps for a certain section of the population. One character with which correlation was thought possible is thallus size. Thus large, medium, or small plants may have been consistently convolute. Figure 7 shows the three types of convolution divided into their respective size classes, against the total number of plants in each. 82% of the population had a thallus length

TABLE 6 - The Correlation Between Thallus Length, Position and Extent of Convolution.

Plant Number	Thallus Length cm	Convolute Basal Region	Convolute Median Region	Convolute Distal Region	Plant Number	Thallus Length cm	Convolute Basal Region	Convolute Median Region	Convolute Distal Region
1	15			X	26	2		X	X
2	7		X	X	27	2.5			
3	10		X	X	28	2			X
4	3.5		X	X	29	4.5			
5	5				30	2.5		X	X
6	7			X	31	5.6		X	X
7	6.5				32	8		X	X
8	3		X	X	33	6.5		X	X
9	3.5		X	X	34	4.5		X	X
10	1.5			X	35	4.5			
11	3		X	X	36	3			
12	3.5		X	X	37	4		X	X
13	5				38	4			X
14	3.5			X	39	3.8			
15	4.5				40	4.5		X	X
16	14		X	X	41	3			
17	4		X	X	42	2.5			
18	1.5				43	7			
19	11		X	X	44	2.75			
20	9.5		X		45	5			
21	9.5		X	X	46	5.5		X	X
22	6.5		X	X	47	3			
23	2.5		X		48	6.5			
24	3		X	X	49	6		X	X
25	7		X	X	50	8		X	X

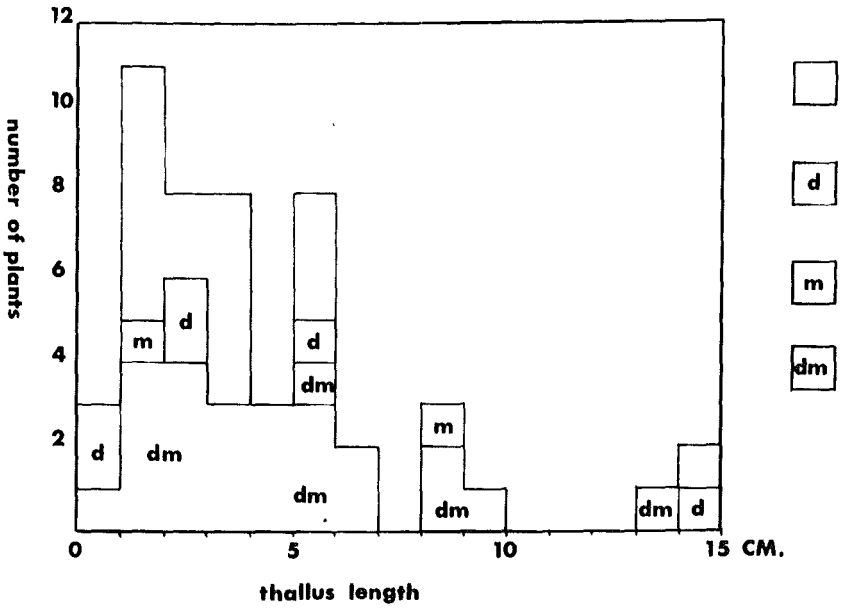


FIGURE 7 - A graph showing the relationship of the type of thallus convolution to plant length, in the Bluff Enteromorpha sample. (d) distal region only convolute, (m) median region only convolute, (dm) both distal and median regions convolute, unmarked areas indicate the absence of any form of convolution.

not exceeding 7 cm.

Only 27 out of the total of 50 plants had convolution extending over both median and distal regions. 24 of these were less than 7 cm in length. Of the total of 6 plants with more restricted distal region convolution, only one had a length greater than 7 cm, and similarly one of the two plants with median region convolution.

Therefore, any of the three types of convolution recognised would be more likely to occur in the longer than 7 cm portion of this population. The ratio of non-convolute to convolute plants for the entire sample was 1:1.63. That for the longer than 7 cm portion was 1:9, and for the shorter than 7 cm portion, 1:1.26.

If the original sample was truly representative of the population, the following may be concluded on the basis of the above evidence. (1) The chances of a plant longer than 7 cm being convolute are greater than those of a smaller plant. (2) This is not a consequence of the smaller number of individuals at the larger end of the size scale. In theory, if the level of comparison was raised from e.g. 7 to 10 cm and the size of the sample increased, the chance of the larger plants being convolute should still remain proportionately greater. (3) While there is a greater chance of collecting a large convolute plant than a small one, the writer considers that (a) the greater likelihood of collecting a small (less than 7 cm) plant and (b) the decreased likelihood of it being convolute render any combination of thallus size and convolution an unreliable taxonomic criterion for this population. (4) The occurrence of distal and median region convolution alone is considered too rare to be of use in the classification of this population.

However, having established that a significant portion of the population was convolute, it is relevant to recount at this point the results of experiments designed to show how the convolutions originated.

The Role of Cell Division in Convolution. According to Fritsch (1935) the thallus in Enteromorpha grows by the activity of an apical cell at the uniseriate stage, which is gradually replaced by intercalary growth in the multiseriate stage. The development of a convolute thallus would appear to result from a localisation of growth in certain regions. Each centre of growth would manifest itself as a small irregular outward projection - a convolution. No study appears to have been made to determine whether these are caused by -

(1) either a greater number of cells in these regions compared with the surrounding ones, due to the longer duration or greater rate of cell division,*

(2) an increase in cell size of the region,

or (3) a combination of both.

The Bluff population constituted ideal material for this study. As the convolutions were relatively large compared with the overall width of the thallus, there was a good chance that the 10 measured cells belonged to a convolution.

---

* A short time after the draft of this thesis was written, a paper by Gayral (1960) was finally obtained. In it, he suggested that 'crimping' of the thallus was brought about by a greater rate of cell division in the effected regions. No experimental work appeared to have been done. The present writer completely disclaims any influence from Gayral in his experimental approach or interpretation of results, because Gayral's paper arrived several months after the draft thesis was written.

If a greater rate of cell division was the cause of convolution of the thallus, then it is likely that more cells in these regions would be in different stages of growth. It would be expected therefore that they would have the greatest variations of regional cell diameter means, from those of the whole population. This is in fact what was found. (Figure 8 shows graphically the variation of the 50 median and distal regional means from the population mean.)

The average regional variation of the non-convolute plants from the population median region mean was .22μ, and that of the convolute plants .42μ. In the distal region the average non-convolute variation rose to .69μ and the average convolute variation to .84μ.

A similar comparison of average cell diameter failed to show any significant difference between convolute and non-convolute regions. Therefore, the possibility that a combination of increased speed of cell division and increase in cell size caused the development of convolutions, was not investigated.

Granted that a greater variation from the mean cell diameter is indicative of a greater rate of cell division, the following may be concluded regarding the role of cell division in convolution.

(1) There is a correlation between convolution and a greater rate of cell division.

(2) It appears likely that an increased rate of cell division is a factor contributing to the development of convolution.

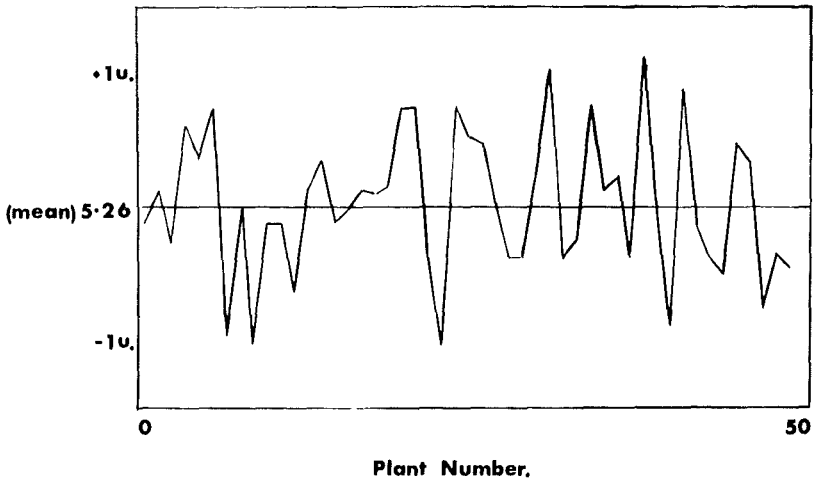
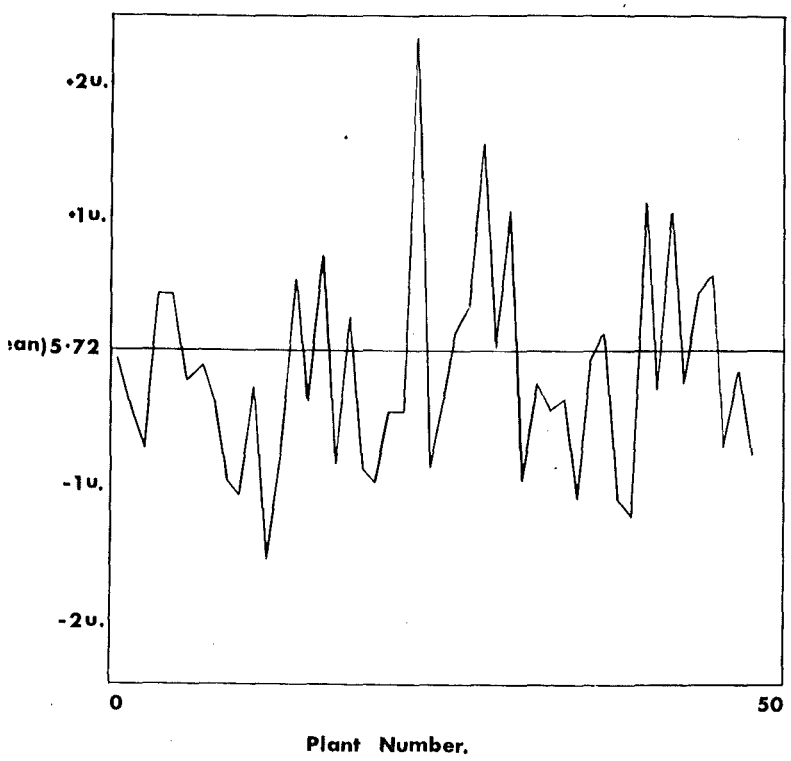


FIGURE 8 - Graphs showing the variation of the individual median region (above) and distal region (below) cell diameter averages from their respective averages for the entire Bluff sample.



The Occurrence of Convolution in an Enteromorpha Population at Motunau.

The discussion up to this point has established that little taxonomic importance may be attached to convolution in one population (Bluff) at a specific time during the growing season (January). It is therefore in order to determine whether any importance may be attached to this character at a specific time of the growing season of at least one other population. The following series of observations pertain to an Enteromorpha population growing in the Motunau River, North Canterbury, (Figure 9) during the summers 1964-65 and 1965-66.

The following types of branched plant were collected from point A on 7 November, 1965. (A) Plants with large medium and small convolute and non-convolute branches on the same thallus (Figure 10), (B) Plants with expanded branched non-convolute thalli (Figure 11), and (C) Plants with narrow branched non-convolute thalli (Figure 12). Various degrees of convolution were also found amongst the unbranched specimens of this population (Figure 13).

On the basis of this evidence it appeared although convolution was of so variable occurrence that little taxonomic significance could be attached to it in at least two Enteromorpha populations.

The causes of Convolution.

Comparison of (1) plants collected during successive seasons at the same locality and (2) over a range of the environment during one season at the same locality, suggested that the potential for development of convolution varied with the season, rather than habitat.

FIGURE 9 - A view of the Motunau River, North Canterbury, showing the collecting points established for the purposes of this study

FIGURE 10 - A plant from Motunau bearing large, medium and small convolute and non-convolute branches.

FIGURE 11 - A plant from Motunau with an expanded non-convolute thallus.

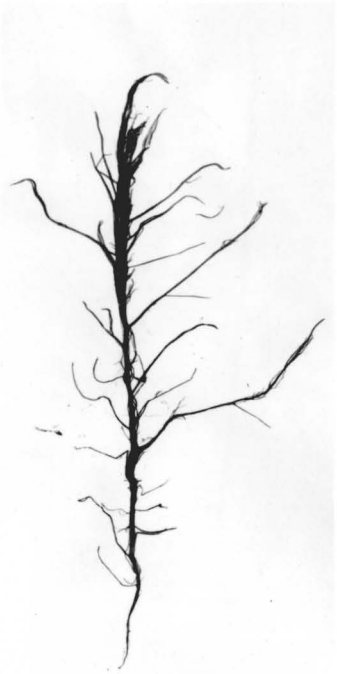


FIGURE 12 - A plant from Motunau with many non-convolute branches.

FIGURE 13 - Unbranched convolute and non-convolute plants of various sizes, from Motunau.

FIGURE 14 - A series of branched and unbranched plants collected during the summer of 1965 from point A, Motunau.

FIGURE 15 - A series of unbranched convolute plants collected from point A, Motunau during the summer of 1966.

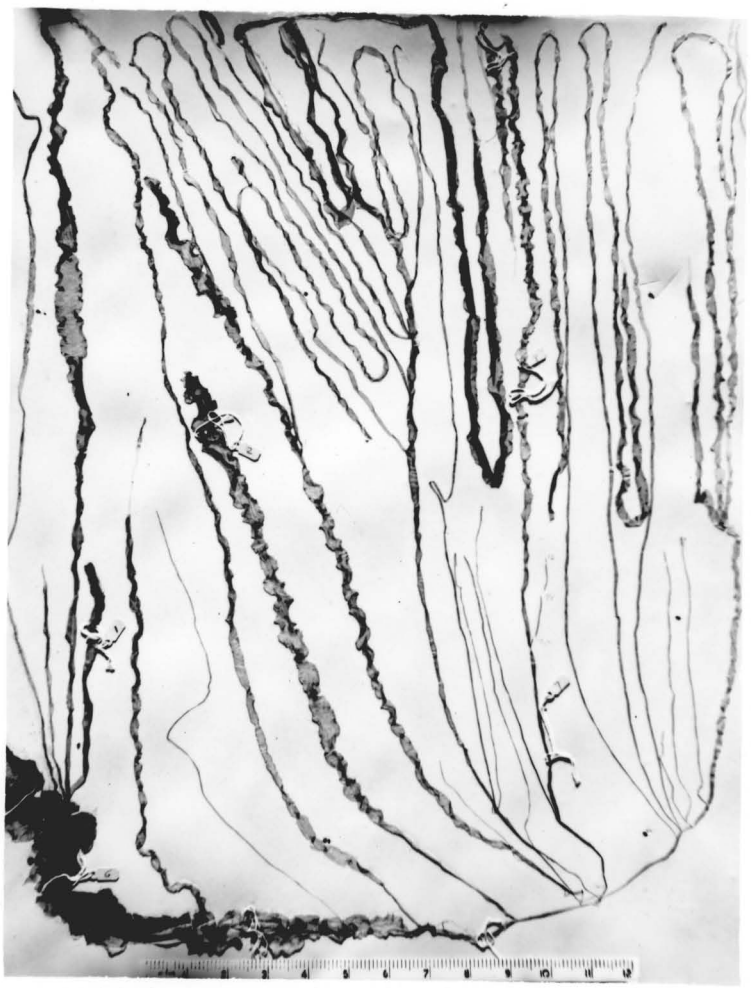


FIGURE 16 - A branched convolute plant of Enteromorpha intestinalis collected from point E, Motunau.

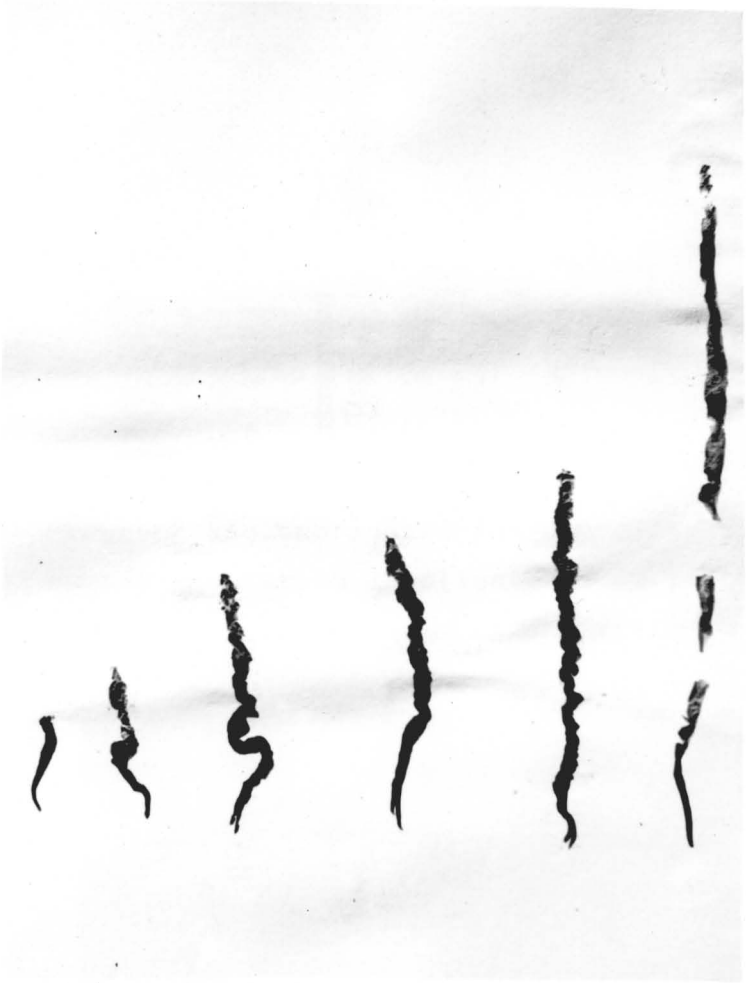


FIGURE 17 - A selection of unbranched convolute plants collected from AA during the summer of 1966.

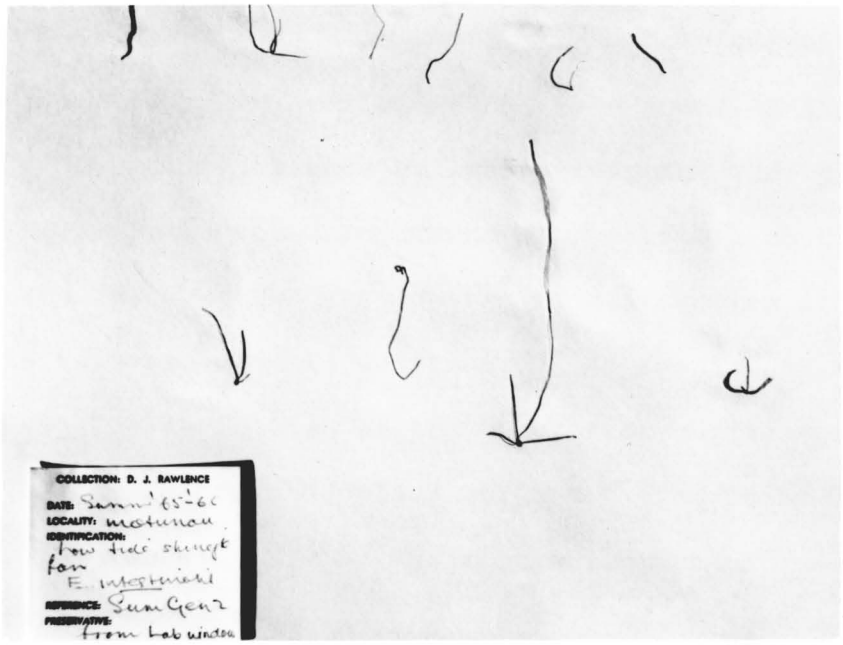


FIGURE 18 - Branched and unbranched non-convolute plants of summer generation 2 collected from point A, Motunau.

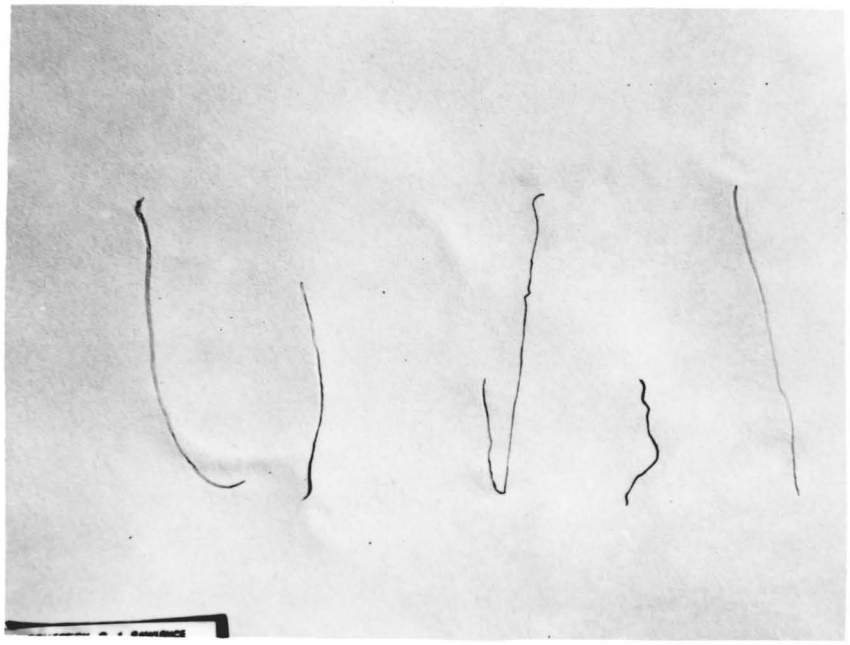


Figure 14 shows a series of plants collected during the summer of 1964-65 from point A (Figure 9). Both convolute and non-convolute branched and unbranched plants of various sizes were found. Figure 15 shows a selection of unbranched plants collected from the same place during the following summer, 1965-66. There can be little doubt that the environment there favoured the development of convolution at the same time during successive seasons. In addition, these conditions existed over the whole range of distribution of the population during the 1965-66 summer. Figure 16 shows a branched plant collected from point E on 7th November 1965, the lowest limit of the population, and Figure 17 a selection of plants collected from AA (Figure 9) in March 1966 the highest limit of the population. Clearly, the conditions favouring convolution existed early in the summer growing season, September - March; and extended over the complete distribution range of the population at least by the end of summer.

On 21 December 1965 identifiable stones were placed in the Motunau River * at A, in order to collect material for embryological investigation. By 27.2.66, a small stand of Enteromorpha plants had become established and reproductive. During the same period an extensive growth of the same type appeared on a large number of the surrounding stones. These plants differed from those established at the beginning of the summer,

---

* More refined methods, such as microscope slides attached to various forms of permanent attachment were originally used for this experiment. However, these were repeatedly stolen or broken by local residents.

which were also reproductive at this time, in that they were considerably smaller and entirely non-convolute (Figure 18). They formed part of a significant growth which appeared approximately half way through the summer growing season, referred to as summer generation 2 to distinguish them from the older plants, established several months earlier.

It thus appears that, although the conditions favouring the development of convolution exist throughout the summer, only summer generation one becomes convolute. It is possible that the other summer generation does not grow long enough, that it does not grow large enough, or that it simply does not possess the capacity to develop convolution. Some indication of the possible cause of convolution may be obtained from a comparison of summer and winter generations growing at Motunau.

Figure 19 shows a selection of winter generation plants collected from point D (Figure 9) on 20 June, 1965. No convolute winter generation plants were collected on this or any other occasion. It has been suggested that the cause of convolution may be a temperature increase (Bliding, 1963). Certainly this appeared to be the main difference between the summer and winter environment at Motunau. The average winter water temperatures (May 30 - September 9, 1965) were $49^{\circ}F$ max. and $39.8^{\circ}F$ min. No summer record was available due to continual theft of research equipment, but the temperatures would certainly be higher than in the winter.

Experimental induction of Convolution

The object of the following experiment was to determine whether convolution could be induced in the winter generation by an artificial rise in water temperature.

FIGURE 19 - A selection of winter generation plants from point D, Motunau.

A number of winter generation plants were collected from Point A, Motunau on September 12, 1965. These were transferred to the laboratory, washed thoroughly in autoclaved sea water to remove most other organisms, and placed in a 500 ml. beaker of autoclaved sea water at the laboratory window. Periodic additions of water were necessary over the next few months to compensate for the minor amount of evaporation. A temperature record was kept during the period September 12 to November 14, 1965.

The maximum attained was 77.1°F. However, it was possible that the temperature of the surface layer exceeded this average quite considerably. A number of large and small plants at the surface became expanded, filled with gas, and convolute similar to the summer generation. Generally those plants below the surface, particularly near the bottom of the beaker, remained unchanged. Figure 20 shows a selection of large and small convolute and non-convolute plants of the winter generation. All were subjected to the treatment detailed above, with the exception that the convolute plants became restricted to the surface (probably considerably elevated temperature) zone.

This evidence supports the view that higher summer temperature is an influencing factor in the development of convolution in the Summer Enteromorpha generation at Motunau. It is the writer's view that this is not the complete explanation. It is likely that the Summer water temperature even in the surface zone of the Motunau River does not reach 77°F. In addition, most convolute plants grow in the lower mid littoral zone, and are exposed only at low spring tides. They could not therefore be subjected to high temperatures through exposure at low tide.

Plants growing in the sublittoral zone, in which temperatures would be considerably less than 77°F, also became extensively convolute.

Figure 21 shows a rock bearing two long plants, one dark and one light green.* The former belonged to the sublittoral, and the latter the mid littoral zone. Both are extensively convolute.

The difference between average summer and winter water temperature at Motunau is probably no greater than 20°F. Convolution therefore develops in the natural environment over a smaller temperature range than in the above experiment. It may be concluded that temperature is not the only factor influencing the development of this character.

General Conclusions. No more than passing mention is made of convolution in the literature. No studies similar to the present one appear to have been made. From the evidence presented above the following conclusions may be drawn regarding convolution as a taxonomic character.

(a) Convolution is an unreliable character since it does not develop on all plants of a population, and the convolute plants correlate only with those plants having a greater than normal variation from the mean cell diameter.

(b) It appears likely that an increased rate of cell division is one of the factors contributing to the development of convolution.

---

* The origin of the colour difference is discussed in a subsequent section.

FIGURE 20 - A selection of convolute and non-convolute plants of the winter generation after a period at an artificially elevated temperature.

FIGURE 21 - Refer to text for explanation.

(c) The summer growing season is that most favourable for the development of convolution. The higher summer temperature is, however, only one of the more important factors influencing its development.

Cell Diameter. The average diameter of basal, median and distal regions, together with an average for the entire plant is set out in Table 2.

The average for the 50 basal regions was 4.78μ, median regions 5.26μ, and distal regions 5.72μ. The variation of the mean of the individual regions from those for the median and distal regions of the entire sample is shown in Figure 8.

(1) The larger variations in median and distal regions are believed to be caused by a greater rate of cell division. This correlates well with the distribution of convolution in these two regions. (2) The smaller variations may simply be attributed to a slower rate of asynchronous cell division, characteristic of the intercalary stage of growth. However, some plants have all cells consistently larger than the average and it is likely that this is unrelated to the rate of cell division. (3) It is more probable that a factor is present which causes all cells of these plants to have a consistently high surface/volume ratio (Thomas et al, 1956).

Thus plants number 25 and 42 (Table 2) have averages for all regions significantly above the normal. Chapman (1956) recorded the cells of old parts of the thallus in one species as being significantly (18μ) longer than normal. As the writer wished to draw conclusions

about cell diameter as a taxonomic character, which were meaningful for the Genus throughout the South Island, it was necessary at this juncture to obtain data from additional populations. Therefore the cell diameter of two smaller (20 plant) samples was analysed in the same manner as the Bluff population. One, constituting sample II Table 7, was collected on November 7, 1965 from point e. (Figure 9) Motunau, while sample III Table 8 was collected from point B on March 10, 1966. Both belonged to the same population.

The extremes in cell diameter for each entire sample of this investigation were - 3.00μ and 9.25μ in sample I, 9.25μ and 26.75μ in sample II, and 7.8μ and 23.4μ in sample III. The ranges of 6.25μ, 17.30μ, and 15.6μ respectively are comparable with those of Chapman (1956). However, the writer felt that by recording average diameter figures based upon large numbers of cells from all regions of the plants rather than diameter extremes based on a few cells, this quantitative character could be made considerably more meaningful. Bliding (1963) appears to have done this already to a certain extent, but Chapman (1956) has not made systematic records of cell diameter averages.

To determine the validity of this idea, (1) the average cell diameter was calculated for each sample and (2) the average variation of each region from the population diameter average was ascertained. (The details of the calculations are set out in Table 9). In this way (1) the diameter of a large number of cells (1,500 in sample I, 600 in each of samples II and III) from 3 regions of each plant were taken into account (2) together with the size of their average variation from

the 3 sample means. As a result it was found that any cell measured was likely to vary from the population mean by only .40μ in sample I, .91μ in sample II and 1.55μ in sample III. The increase in values for the latter two samples could be a result of either the larger average cell diameter or the smaller number of cells measured.

It is the writer's view that fluctuations in the speed of cell division cause the usual variations from the mean cell diameter of the population. It seems more likely that a higher surface / volume ratio causes all the cells of a plant (e.g. plants 25 and 42, sample I) to be larger than normal rather than old age.

This survey therefore supports the conclusion that many New Zealand Enteromorpha populations may have a small range of average cell diameter. The seemingly large variations, presumably of individual cells, quoted by Chapman (1956) e.g. E. clathrata, are within the range of probability as indicated by the present study. Bliding (1963) has demonstrated a larger range of individual cell diameters in E. clathrata than quoted by Chapman. Bliding additionally supports the conclusions reached by the present writer for samples II and III, that the average diameter may decrease from base to apex.

Neither these nor any other workers have disproved the writer's contentions that (a) the range in average cell diameter of a significant sample of any Enteromorpha population is likely to be small and (b) that cell diameter could be a useful taxonomic criterion.

The reason that at present it cannot be used as such lies in the fact that to date the present study appears to be the only one of its nature. Chapman (1956) quoted 47 sets of diameter extremes but few

TABLE 7 - Sample II

Plant Number	Average Cell Diameter						Variation from the regional mean		
	Basal Average	Median Average	Distal Average	Average (plant)	Decrease	Increase	Basal Region	Median Region	Distal Region
1	14.35	12.90	14.55	13.43		*	-02	+ .46	+2.15
2	16.48	13.80	12.76	14.35	*		+2.11	+1.36	+ .36
3	16.95	12.40	11.87	13.74	*		+2.58	- .04	-53
4	12.75	13.27	12.40	12.80	*		+ .38	+ .83	
5	15.34	11.87	12.40	13.20	*		- .04	- .57	
6	13.27	10.08	12.40	11.92	*		- .10	-2.36	
7	16.27	12.57	12.04	13.63	*		+1.90	+ .13	-36
8	11.20	11.70	12.40	11.77		*	-3.17	- .74	
9	15.72	12.53	11.70	13.31	*		+1.35	+ .09	- .70
10	14.68	12.58	12.00	13.09	*		+ .31	+ .14	- .40
11	13.45	13.10	11.18	12.58	*		- .92	+ .66	-1.22
12	15.15	12.77	11.70	13.21	*		+ .78	+ .33	- .70
13	16.23	12.05	12.05	13.44	*		+1.86	- .39	- .35
14	15.20	11.75	11.70	12.88	*		+ .83	- .69	- .70
15	14.48	11.88	12.23	12.86		*	+ .09	- .56	- .07
16	17.65	11.70	11.16	13.50	*		+3.28	- .74	- .24
17	13.10	13.63	12.63	13.12	*		-1.27	+1.19	+ .23
18	11.70	12.80	11.88	12.13	*		-2.67	+ .36	- .52
19	10.90	12.59	16.65	13.38		*	-3.47	+ .15	+4.25
20	12.65	12.75	12.40	12.60	*		-1.72	+ .31	
	Regional Averages			Whole Plant Average			Average Variation		
	14.375	12.44	12.405	13.07			1.49	.605	.64

TABLE 8 - Sample III

Plant Number	Average Cell Diameter			Average (plant)	Increase	Decrease	Variation from the regional mean		
	Basal Average	Median Average	Distal Average				Basal Region	Median Region	Distal Region
1	16.25	13.59	15.28	15.04		*	-2.8	-2.24	-1.38
2	15.30	13.81	14.70	14.60	*		-3.75	-2.02	-1.96
3	22.02	15.33	14.23	13.89		*	+1.97	- .50	-2.43
4	16.56	15.99	14.21	15.58		*	-2.49	+ .16	-2.45
5	19.46	14.80	15.49	16.58		*	+ .41	-1.03	-1.17
6	19.49	16.48	16.96	15.31		*	+ .44	+ .65	+ .30
7	16.28	13.84	16.16	15.42		*	-2.77	-1.99	- .50
8	15.68	16.16	17.53	16.45	*		-3.37	+ .33	+ .87
9	19.68	16.17	20.65	18.86	*		+ .63	+ .34	+3.99
10	22.60	15.58	17.57	18.91		*	+3.55	- .25	+ .91
11	20.47	17.93	19.28	19.22		*	+1.42	+2.90	+2.62
12	17.34	17.54	17.15	17.34		*	-1.71	+1.70	+ .49
13	20.05	19.10	17.04	18.73		*	+1.00	+3.27	+ .38
14	21.63	15.19	18.41	18.74		*	+5.28	- .64	+1.75
15	16.55	15.78	15.00	15.77		*	-2.50	- .05	-1.66
16	19.08	16.16	17.14	13.12		*	+ .03	+ .33	+ .48
17	20.93	14.99	16.36	17.42		*	+1.88	- .84	- .30
18	20.66	16.16	15.77	17.53		*	+1.61	+ .33	- .89
19	19.89	17.13	12.65	13.22		*	+ .84	+1.30	-4.01
20	21.05	14.99	11.88	15.97		*	+2.00	- .84	-4.78
	Regional Average			Whole Plant Average			Average Variation		
	19.05	15.83	16.66	17.18			1.9	1.09	1.67

The data on the facing page was prepared as follows:-

(1) Average cell diameter for basal, median, and distal regions. This was calculated by averaging the 10 cells of e.g. the basal region of every plant. The 50 figures were themselves averaged giving a figure for the basal regions of the 50 plants.

(2) Variation for basal, median, and distal regions. The 10 cells of each region were averaged, and the individual deviation from the mean of the 50 or 20 basal regions of the sample calculated. The total difference of e.g., the basal regions was calculated and averaged giving the figure for 'variation'.

(3) Range. The range was calculated as the difference between the largest and smallest 'Average Cell Diameter'.

The procedure was repeated for the median and distal regions, and the mean of the 3 taken as the average for the whole plant.

TABLE 9 - Summary of Cell Diameter Data Derived from Samples I, II, and III. (Tables 2, 7, and 8).

Sample I - Plants Collected from the Upper Littoral Zone, Bluff (50 Plants)			
Average Cell Diameter : Basal Region	4.78	Average variation	.31
" " " : Median Region	5.26	Average variation	.23
" " " : Distal Region	5.72	Average variation	.67
Range of Average Cell Diameter	.94		
Average Cell Diameter : Whole Plant	5.23		
Sample II - From Point C Motunau River (20 Plants)			
Average Cell Diameter : Basal Region	14.38	Average variation	1.49
" " " : Median Region	12.44	Average variation	.61
" " " : Distal Region	12.41	Average variation	.64
Range of Average Cell Diameter	1.9		
Average Cell Diameter : Whole Plant	13.18		
Sample III - From Point B Motunau River (20 Plants)			
Average Cell Diameter : Basal Region	19.05	Average variation	1.9
" " " : Median Region	15.83	Average variation	1.09
" " " : Distal Region	16.66	Average variation	1.67
Range of Average Cell Diameter	2.3		
Average Cell Diameter : Whole Plant	17.18		

averages for the New Zealand taxa. It does not appear necessary for the excessively large or small cells to be included in type descriptions. In the present investigation, large cells were consistently associated with the localised region adjacent to the stipe. Here they are influenced by the stimulus responsible for rhizoid formation. More distal smaller cells maintain a delicate balance with this stimulus (discussed more fully in a subsequent section). Where the equilibrium is occasionally upset and a large cell produced, such an abnormality can be justly ignored.

In order to utilize cell diameter as a taxonomic aid in the identification of the Bluff sample, it was necessary to compare an average figure with those of Chapman (1956). Therefore an average figure was compared against a range delimited by the extremes of diameter recorded for each taxonomic unit. It was not surprising therefore that the diameter of the Bluff population fell within the range of 14 of Chapman's (1956) taxonomic units.

Womersley (1956) draws attention to one possible explanation for these large ranges. With regard to an Enteromorpha collected by himself and identified by Chapman as E. bulbosa (Suhr) Montagne 1846 (1949 : 496 Figure 3) Womersley states "Chapman's description is not accurate: the main thallus is scarcely compressed and (1) his measurements are faulty and (2) some apparently misprinted . Bliding regards these specimens as a form of E. clathrata with which I agree." This is an example of misidentification of a specimen caused by (1) inaccurate observation of morphological features and (2) inaccurate measurements of cell diameter.

In most plant groups a combination of other characters would reduce the Bluff population to a species. As will be demonstrated in the following sections, these characters are too variable to use in Enteromorpha. The writer is not satisfied that with the difficulty of classification created by this situation, the following have not occurred:-

(1) Specimens may have been mistakenly identified as a certain species with the aid of a combination of characters excluding cell diameter,

and (2) that the cell diameter range for that species has not been increased to accommodate larger celled specimens identified in this way.

It is worthy of note at this point that many algal taxonomists are most reluctant to investigate the systematics of the Ulvaceae. As a result it has long remained a neglected family (Papenfuss, 1958).

Summary - Conclusions - Cell Diameter. The three populations investigated had small cell diameter ranges compared with those of Bliding (1963) and the few quoted by Chapman (1956). The apparent discrepancy may be caused by a difference of approach - no studies similar to the present having been made. Other contributing factors appear to be (1) the result of specimens being incorrectly placed in a species on grounds other than cell diameter and (2) faulty measurements, Womersley (1956).

As a result, it is not possible at present, to use cell diameter alone to identify a specimen as belonging to even a small group of

species, or to use a combination of other characters* to identify a single species. Thus we find ourselves in a taxonomic quandary alluded to by Chapman (1956), Womersley (1956), Bliding (1963) and others.

However, the writer believes that if more extensive studies similar to the present one were made, cell diameter could become a useful character.

* Discussed in subsequent sections.

Chloroplast Morphology and Orientation. It is convenient to discuss these two characters concurrently, as they are simply aspects of the one structure although treated by various authors as independent taxonomic criteria. Chloroplast colour is considered in a separate section. However, to avoid repetition later, it was found necessary to mention it in the present section.

The size of the chromatophore has been used in the delimitation of Enteromorpha spp. since the time of J.G. Agardh (1883). E.g. Setchell and Gardner (1920, P.247) distinguished between E. crinata and E. erecta, in which the cells were filled by the chromatophores and E. plumosa and E. clathrata in which they were not. However, Bliding (1944) showed that the chloroplast of a single species could be environmentally modified. Abundant food and shade caused the development of large dark green chromatophores.

In addition, he showed that cells in various positions on the thallus - ranging from apical cells to those at the base of the blade - had a different chromatophore structure.

This variability may well have engendered the rather variable forms of expression applied to different types of chloroplast morphology by Bliding (1963). On P.7 of this Paper, E. clathrata is stated to have a disc shaped chloroplast, while on P.109 it is referred to as a laciniate faintly vaulted disc within the outer wall. Both statements referred to Fig. 67d, and would not be mutually exclusive if they referred to the chromatophore viewed in different planes. Neither of these terms adequately describes the chromatophores figured for the same species in Bliding (1955).

In addition, Bliding appears to have differentiated between chromatophores differing solely in the number and mode of occurrence of their pyrenoids. The chromatophores of E. clathrata (Fig. 67d. 1963) and Blidingia (Fig. 14c. P.38, 1963) apparently differ only in this character, yet the chloroplasts in the latter are termed stellate. Fritsch (1935) pointed out that the pyrenoids of green algae may both appear and disappear in response to a variety of environmental conditions.

In view of the variability of the chromatophore which Bliding has shown, it hardly seems necessary to use mutually exclusive terms for the chromatophores of E. clathrata unless it is stated that they refer to different planes of view. For the same reason, it is unwise to differentiate the same general form of chloroplast occurring in separate genera (E. clathrata and Blidingia) by distinct terms. It could be argued that with a moderate degree of variation in one or both groups, terms intended as differentiae were only synonyms.

In brief, Bliding's chromatophore terminology is loose, and therefore his summary of types occurring in the Ulvaceae, in the light of his own work, is rather misleading.

Summary of chloroplast types found in the Ulvaceae, after Bliding (1963).

(a) Blidingia 'stellate'

(b) Enteromorpha flexuosa 'More or less cuff formed with denticulate margins'.

(c) E. clathrata 'disc shaped in the main stem'.

(d) The E. intestinalis group of species 'hood-like'.

(e) E. ramulosa 'a vaulted denticulate disc, covering the inside of the outer cell wall or one of the side walls'.

The terminology applied by Bliding (1963) and Chapman (1956, 1961) to the same species of Enteromorpha appears to be divergent. According to the latter (1956) all 6 New Zealand taxa of E. clathrata and the 5 Jamaican taxa recognised (1961) had granular chloroplasts.

Bliding (1963) describes the chloroplast of E. intestinalis as hood-like. Chapman (1956, 1961) states simply that it fills the cell. It is not possible to determine chloroplast morphology accurately from the latter's diagrams. However, they suggest a structure for E. intestinalis similar to the granules of E. clathrata.

The plastid in fully grown cells of E. ramulosa is described as 'mostly a vaulted denticulate disc, covering the outer cell wall or one of the side walls' (Bliding, 1963). Chapman (1956) described the morphology of the New Zealand E. ramulosas as homogeneous, often completely filling the cell. In view of the variation already discussed it is difficult to know whether these workers are referring to constant heritable types of chloroplast. If so, it is possible that breeding experiments are necessary to determine whether these are differences of geographic races of one species, or independent species.

The following investigations were designed to determine what emphasis could be placed on chloroplast morphology as a taxonomic criterion in a single population.

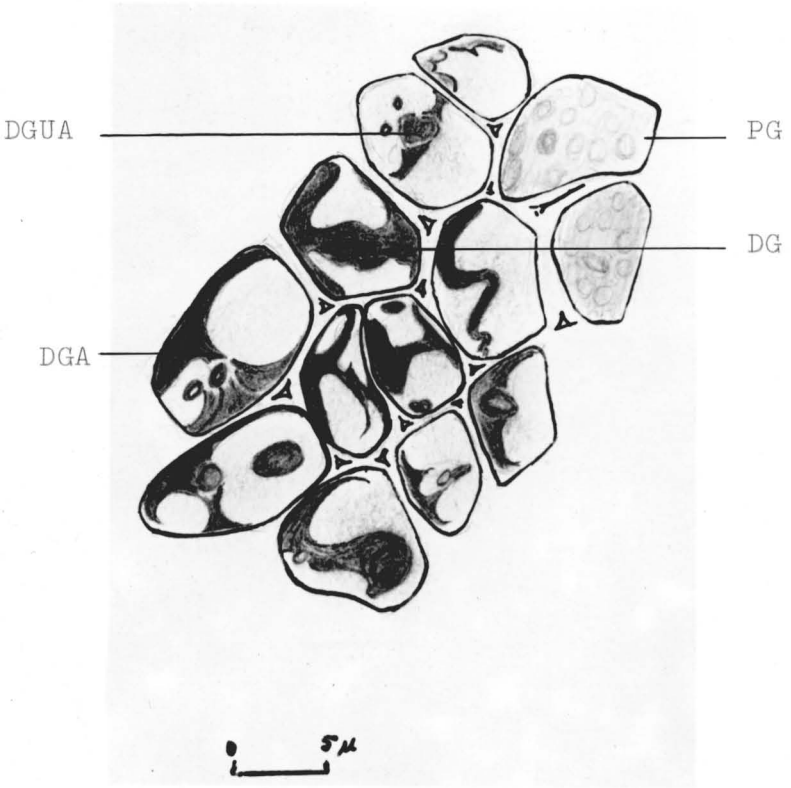


FIGURE 22 - There are both pale and dark green chloroplasts in the above region. Some of the latter are clearly attached to a cell wall, others not. The chloroplasts lack any constancy of form or orientation. PG; pale green: DG; dark green: DGUA; dark green chloroplast not attached to any wall: DGA; dark green chloroplast attached to a cell wall.

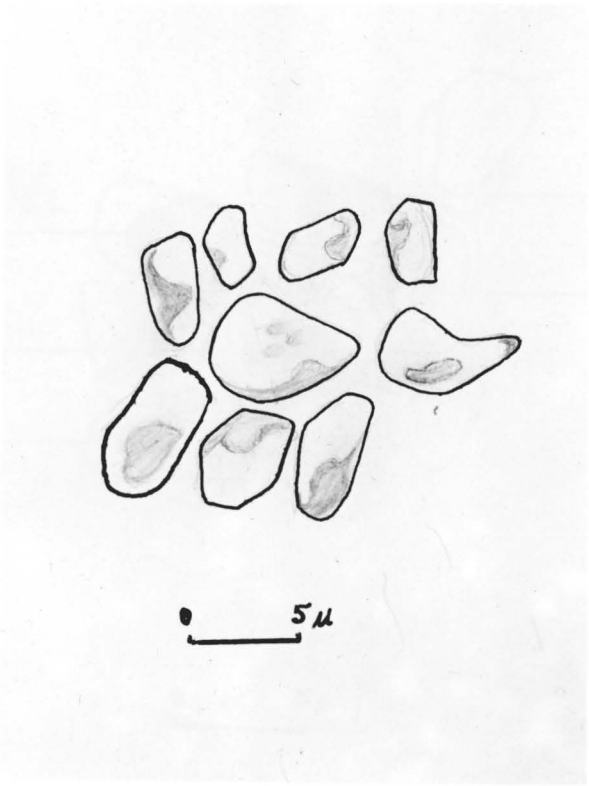


FIGURE 23 - The chloroplasts in this region are all pale green, attached to one wall only, and completely lacking in pyrenoids.

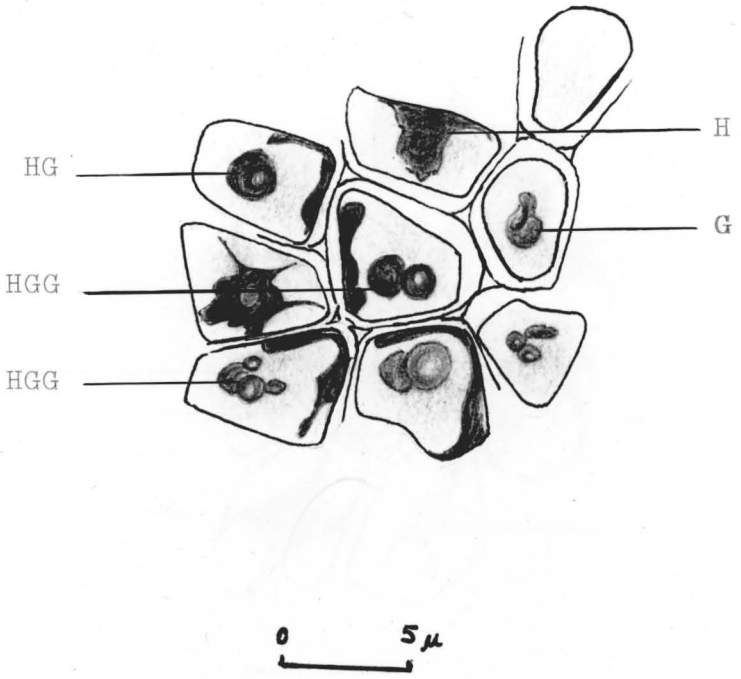


FIGURE 24 - There are two types of chloroplast in the above region - homogeneous and granular. The latter type may comprise one to several granules.
H; completely homogeneous chloroplast:
G; completely granular chloroplast:
HG; homogeneous chloroplast with a single granule:
HGG; homogeneous chloroplast with several granules.

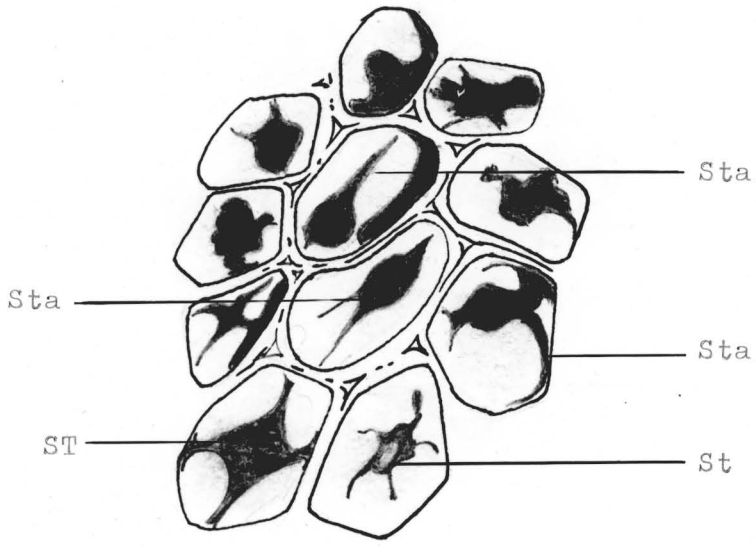


FIGURE 25 - The stellate chloroplast occurred in many plants. In the above diagram several developmental stages of this type are visible. ST; well developed stellate chloroplast attached to the cell walls by one or more arms: St; poorly developed stellate chloroplast, not attached to any wall: Sta; stellate chloroplast with one or two well developed arms.

The latter type was frequently so orientated that it appeared to bisect a cell.

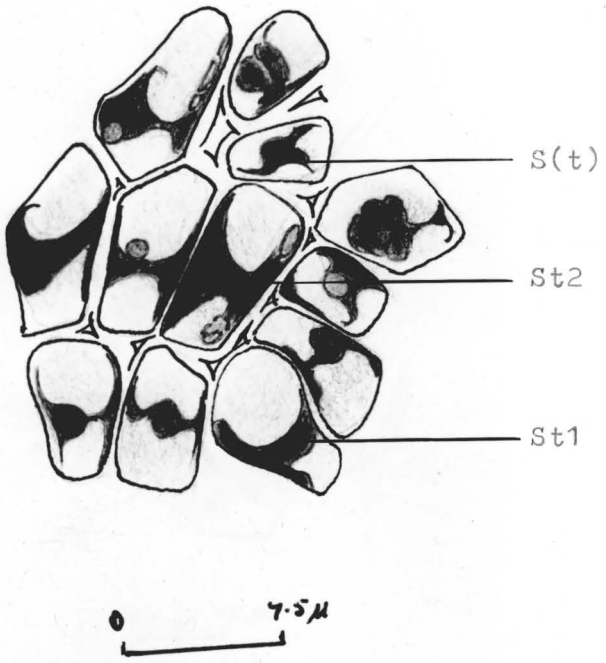


FIGURE 26 - This figure shows another development of the stellate chloroplast. The number of arms has been reduced to one, and the bulk of the chloroplast either (1) St1, attached to one cell wall or (2) St2, divided and attached on two opposing walls, connected by the arm.

Some cells still show the signs of their stellate origin. S(t) stellate chloroplast, not attached to any wall, with two arms and an expanded central portion.

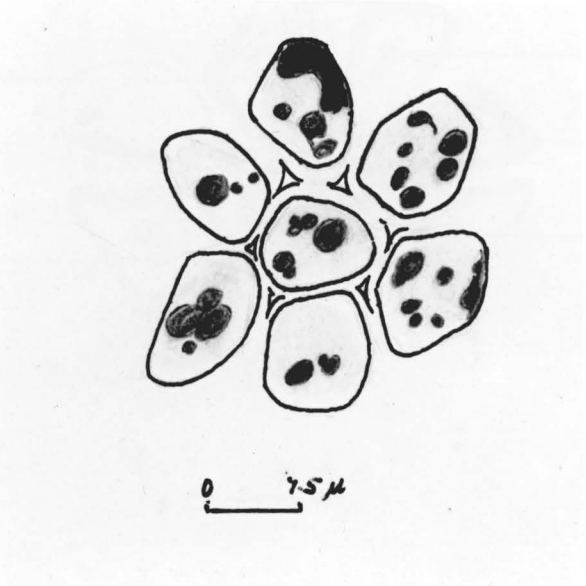


FIGURE 27 - Granular Chloroplasts.

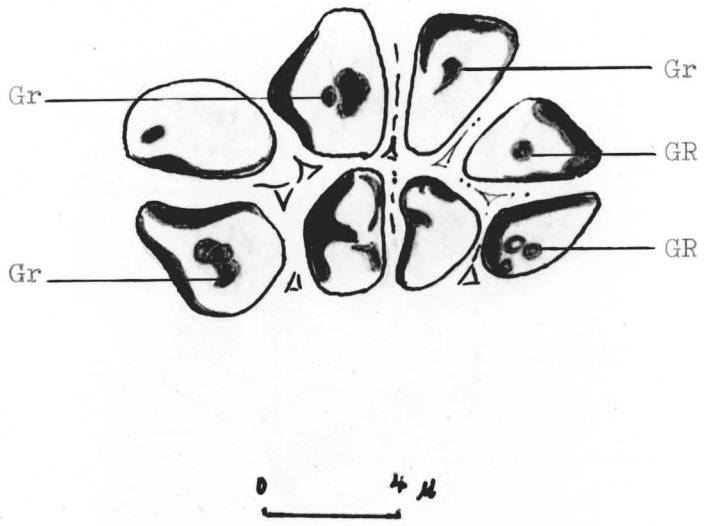


FIGURE 28 - The diagram above shows homogeneous chloroplasts with granular regions. Some granules (GR) are regular in shape, others (Gr) irregular.
The homogeneous region may be attached to any wall, but more frequently that facing the bottom of the thallus, the top of this diagram.

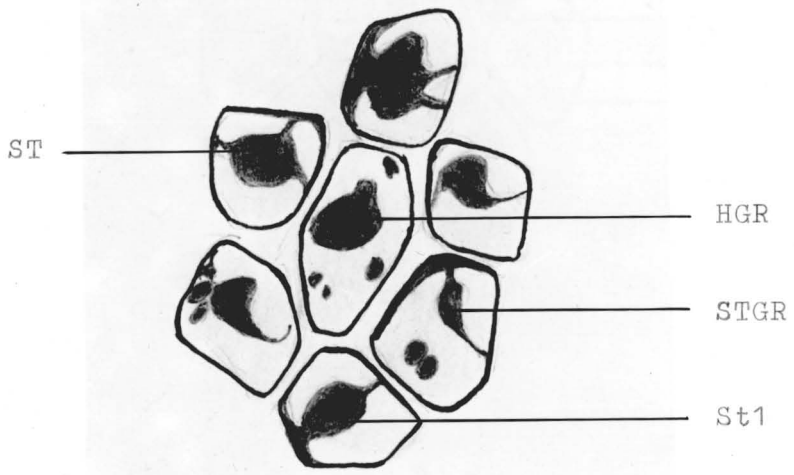


FIGURE 29 - This diagram shows several of the preceeding types of chloroplast in the one region. St; stellate: St1; reduced stellate chloroplast, possessing a homogeneous region attached to one wall, connecting with the opposing wall by a single arm: HGR; a reduced homogeneous chloroplast with several irregular granules: STGR; a reduced stellate chloroplast with several regular granules.

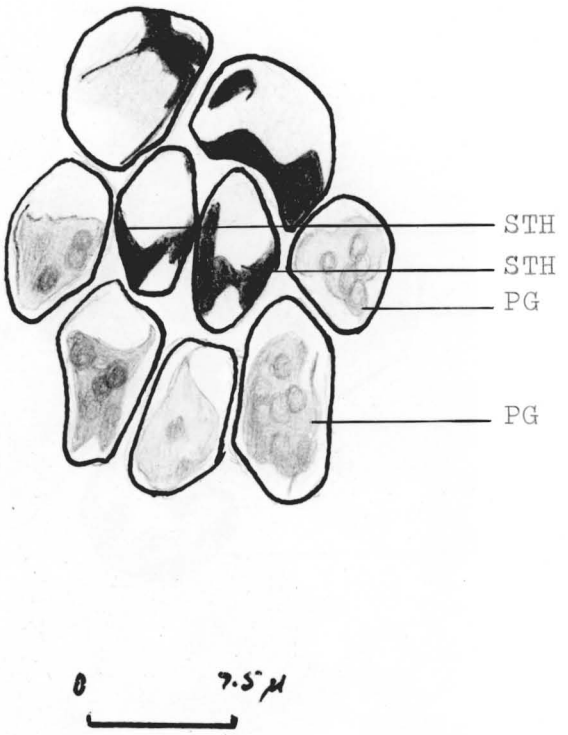


FIGURE 30 - This diagram shows (STH) chloroplasts intermediate in form between homogeneous and stellate. It is not possible to determine them as modified forms of either. Faint granulation is also visible in the pale green chloroplasts (PG).

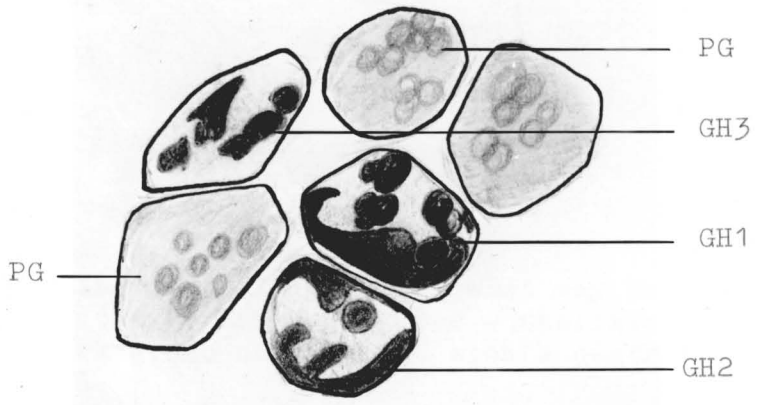


FIGURE 31 - This diagram illustrates the direct relationship between homogeneous and granular chloroplasts. Three stages are visible, GH1 - 3: GH1 shows the homogeneous region still present, GH2 and GH3 its progressive transformation into dark green granules.

Pale green granular chloroplasts (PG) are also present.

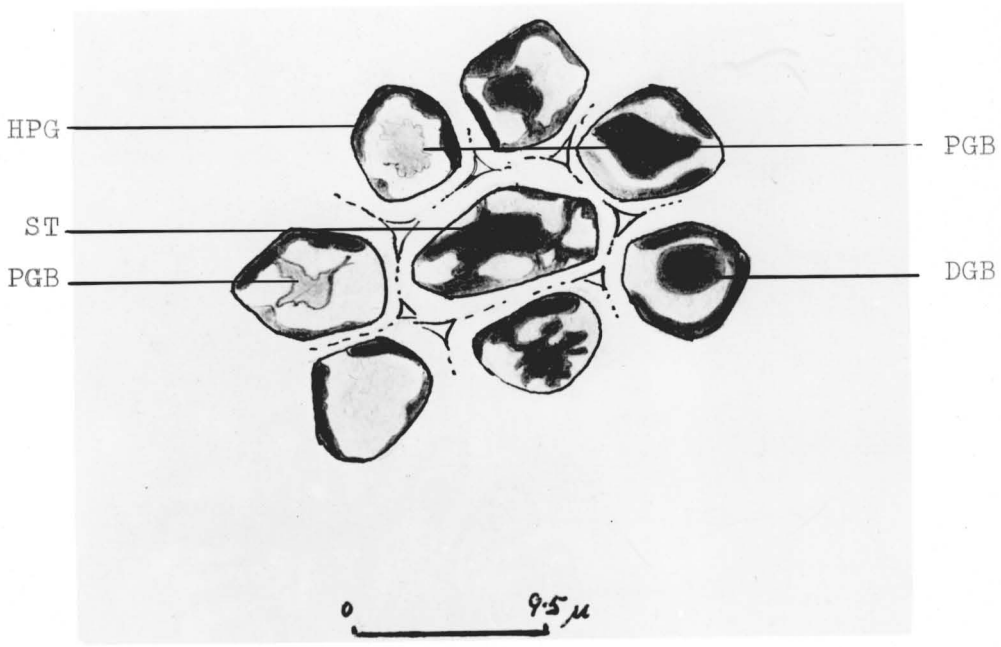


FIGURE 32 - This figure illustrates what may be developmental stages of two chloroplast types - stellate, and the homogeneous dark green chloroplast with a central pale green region HPG.

Development of the Stellate chloroplast. From a dark green chloroplast closely appressed to several walls of the cell, general contraction of the plastid occurs into local regions. Where this occurs toward the outside and centre of the chloroplast a stellate pattern (ST) as above forms. Where contraction is toward the centre alone, a pattern similar to ST and St in Figure 25 results.

Homogeneous dark green / pale green chloroplast. When contraction is localised two distinct regions may develop. The smaller region is abscissed but may remain attached to the wall as a dark green body (DGB), or migrate into the centre of the lumen and become pale green (PGB). The dark green chloroplast with a pale green centre is very common.

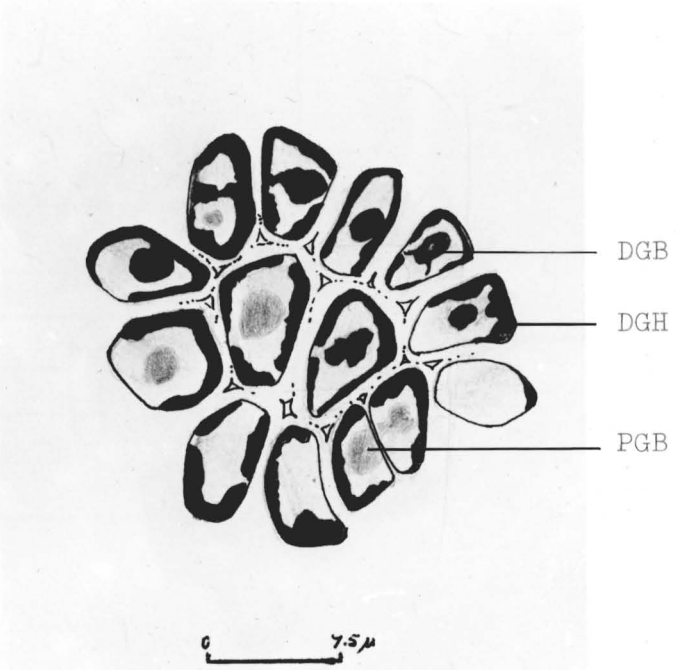


FIGURE 33 - In the above region, the chloroplasts are so orientated that their perimeter coincides with the cell walls in surface view. The cells are therefore fringed with a dark green homogeneous region (DGH) enclosing a dark green (DGB) or pale green (PGB) body. The method of formation of this discrete section of chloroplast is described below Figure 33.

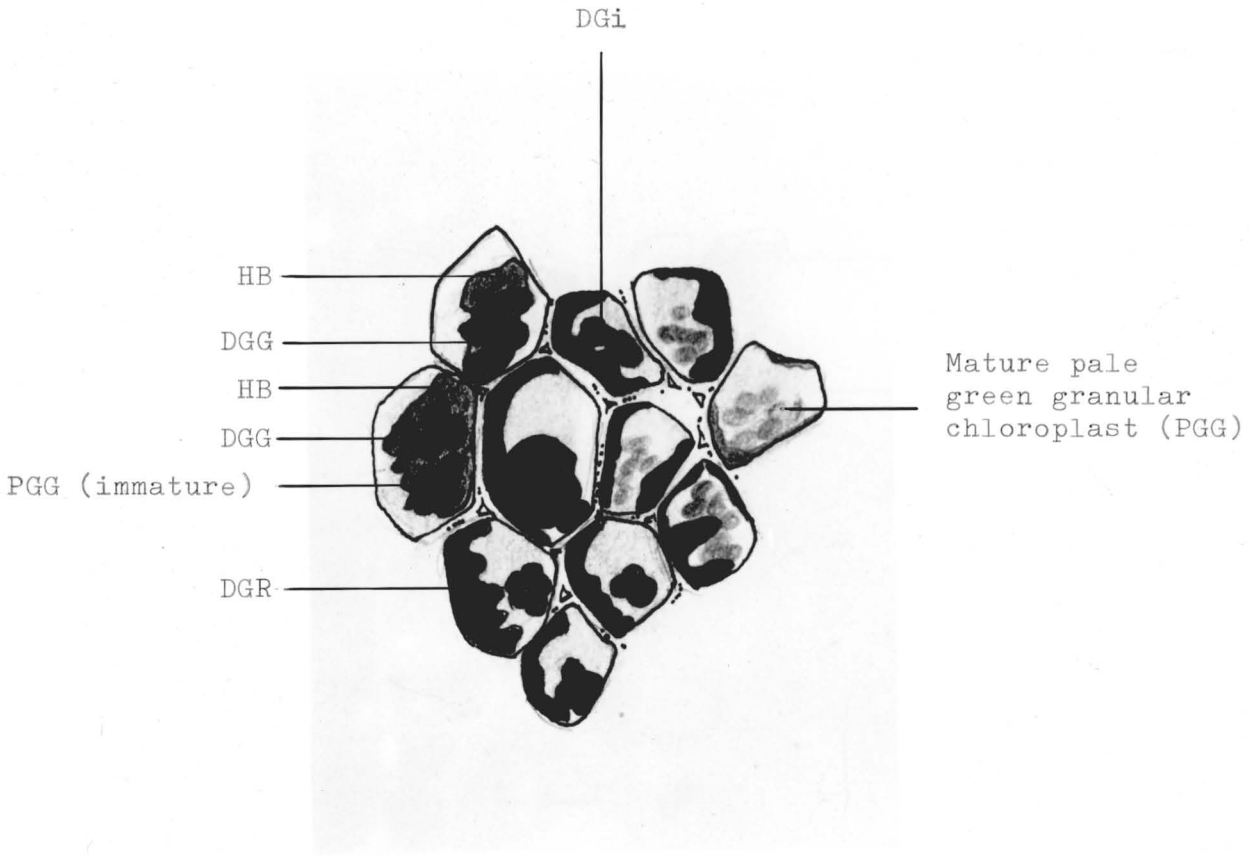


FIGURE 34 - Basal Region of a young plant showing the formation of the following chloroplast types.

1. Pale Green Granular. (PGG) In some cells the granules are still dark green (DGG) with the homogeneous background already pale green (HB).

2. Dark Green Homogeneous Chloroplast with dark green regular (DGR) granules and irregular granules (DGi).

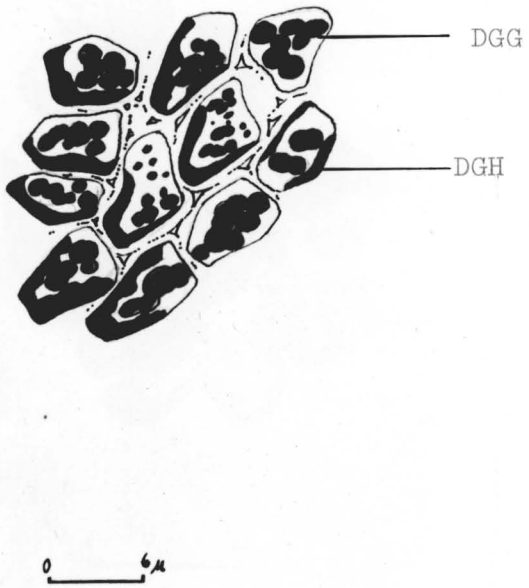


FIGURE 35 - Median Region of a young plant dominated by two chloroplast types - dark green granular (DGG) and dark green homogeneous with granules (DGH).

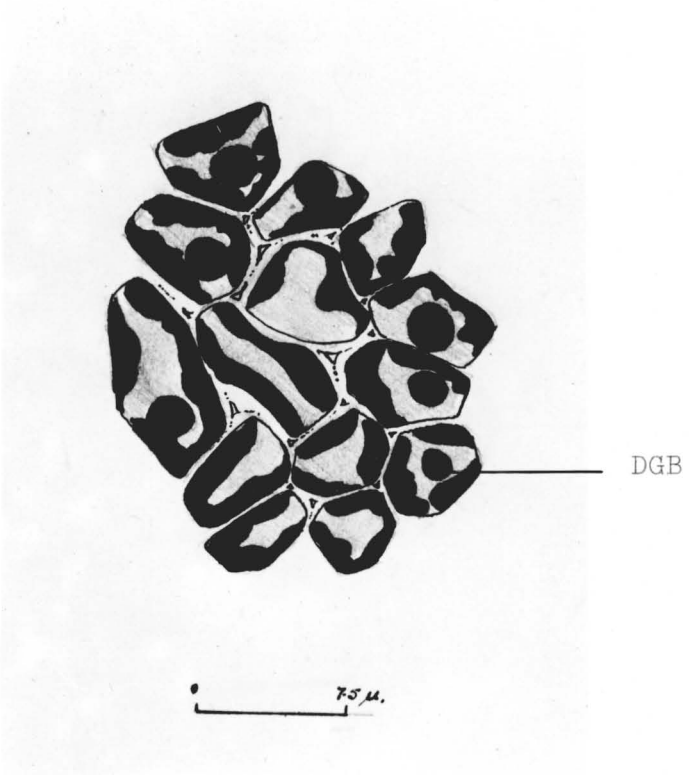


FIGURE 36 - Basal Region of a young plant to illustrate that (1) not all basal regions of small plants have the same chloroplast morphology (compare with Figure 36) and that (2) the homogeneous dark green chloroplast with a discrete dark green region (DGB) may form early in ontogeny.

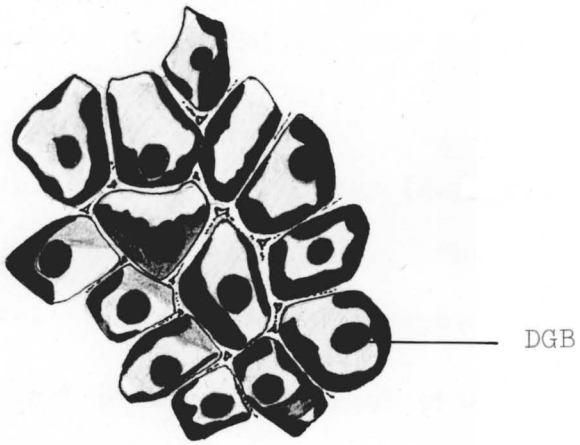


FIGURE 37 - Median Region of a young plant to illustrate that not all median regions of young plants have comparable chloroplast morphology. Compare with Figure 35. The region above is dominated by dark green homogeneous chloroplasts with discrete dark green bodies (DGB).

A study of the variation in chloroplast morphology and orientation in a single Enteromorpha population at a specific time during the growing season. In order to reach more reliable conclusions from the results of this study, a comparison with other populations was included. A similar procedure was adopted in the sections on cell diameter and chloroplast and thallus colour.

Method. The technique for handling this sample has been discussed in an earlier section.

Sixteen types of chloroplast morphology (designated by numbers 1 - 16) were recognised in the present study. These constitute Figures 22 - 37 inclusive. The main types are summarised below.

(a) Homogeneous dark green chloroplast attached or unattached to a cell wall, Figure 22.

(b) Homogeneous dark green chloroplast in combination with one of the following types of granulation:-

1. one large dark green regular granule, Figures 24, 28, 32, and 36.
2. several small irregular granules, Figure 28.
3. several small regular granules, Figure 28.

(c) Stellate, Figure 25.

(d) Pale green granular chloroplast, Figures 22, 23, 30, and 34.

For each of the 150 samples examined (50 each for the basal, median, and distal regions) the number of the chloroplast type which it most closely resembled was recorded. Some samples possessed chloroplasts of two recognised types. In addition, the degree of

correspondence between each sample and the type was recorded. Those not corresponding closely were noted as 'modified'. The detailed results of this survey appear in Tables 3, 4, and 5.

The chief object of this section of the investigation was to determine whether there was any regular variation of chloroplast morphology in the Bluff Enteromorpha sample. It was possible that a regular change may have occurred between basal and distal regions. This was discounted on the following grounds: (1) Of the total 16 chloroplast types, 10 occurred in the basal regions, 11 in the median regions, and 9 in the distal regions. (2) A statistical investigation discussed in Appendix I also failed to show any significant correspondence between thallus size, or certain regions of the thallus, and any of the 16 chloroplast types recognised. From these it may be judged that there was no correspondence between thallus size and chloroplast morphology, and no regular change from basal to distal region in any size class.

On the basis of the diversity of chloroplast types within the population, and within each of the three regions of the individual plants, it is logical that there should be some interrelationship between the various forms.

It was possible to trace a series of changes in various directions from a homogeneous type of chloroplast. The interrelationship of this type with stellate and granular forms is shown in Figure 38 and diagramatically in Figures 39 and 40. The primary formative process is a shrinkage of the chloroplast. This may occur toward the centre and perimeter of the plastid (Figure 32, ST) or the centre alone.

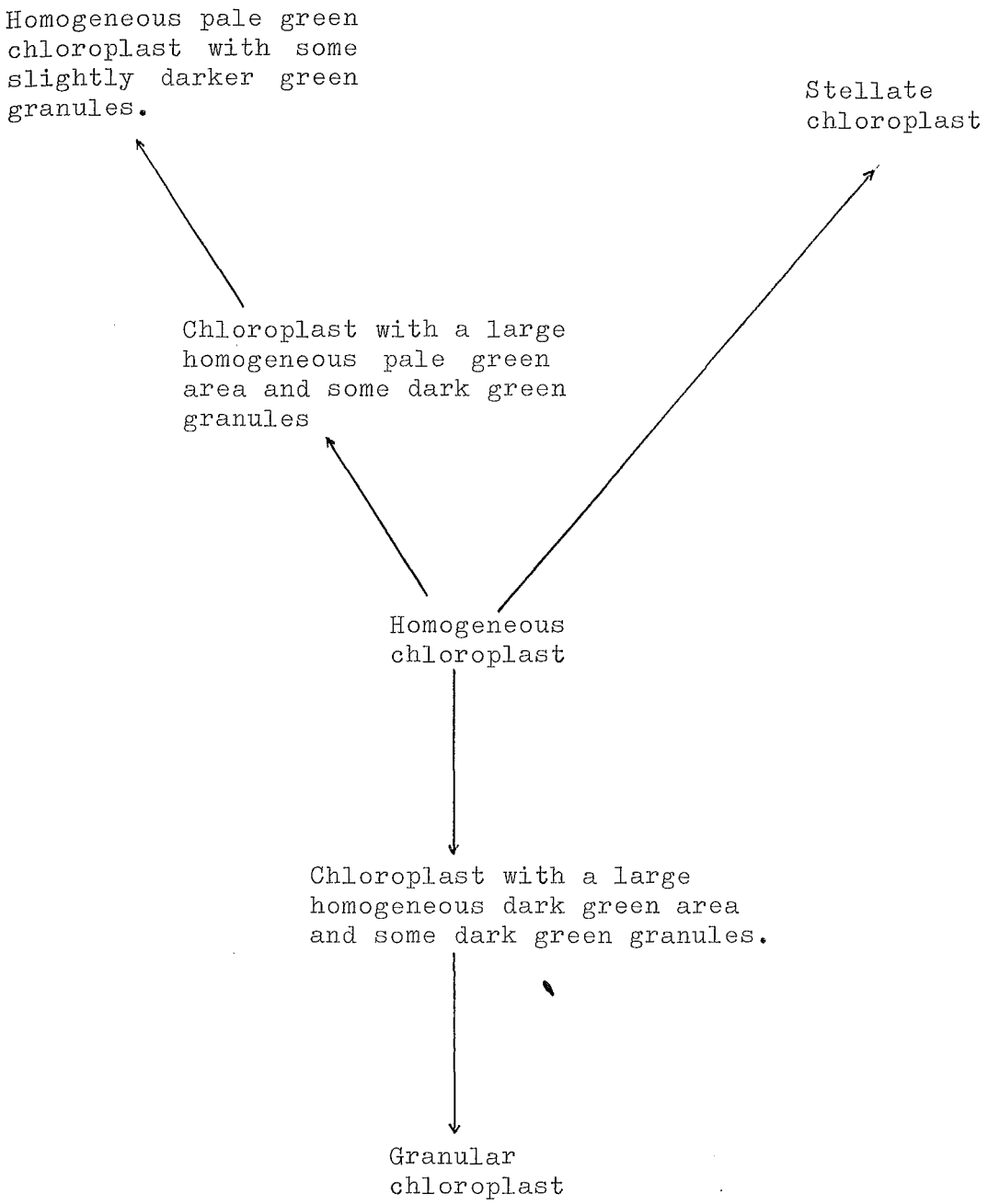


FIGURE 38 - Showing the Interrelationship of the Homogeneous and Other Chloroplasts Recognised in a Population of Enteromorpha at Bluff.

The arms so formed may or may not remain attached to the cell walls (cells labelled ST and St respectively in Figure 25). Shrinkage may, however, be more localised, dividing the plastid into two equal regions attached to opposite walls, which frequently remain interconnected (cells labelled DGA, Figure 22 and STH, Figure 30). On other occasions two unequal regions may be formed. The smaller of these is usually circular, and may remain attached to the wall as a dark green body (DGB, Figure 32) or migrate to the centre of the lumen and become pale green (PGB, Figure 32). This highlights the second formative process, that of physiological change.

Figure 40 shows the formation of dark green granular and pale green faintly granular chloroplasts. Localised shrinkage of the plastid occurs. In some cases no lightening of the colour is evident, even when granule formation is complete (Figures 27, 31, and 40). However, this physiological (colour) change occurs in the sequence of formation of the pale green faintly granular chloroplast. It appears to affect the large homogeneous portion first (Figure 34 and 40) and the granules later to a lesser extent (Figures 31 and 40). The lack of a constant chloroplast orientation throughout the population follows as a corollary to the preceeding situation.

In this population there were a variety of intermediate forms between each of the 16 recognised types. No single form or small group of forms could be considered characteristic of the population. This situation is perhaps comparable to that shown for E. clathrata by Bliding (1944). However, there is no similarity in the amount of variation in the two populations.

Bliding (1963) and Chapman (1956, 1961) make only general reference to chloroplast variation.

The former noted the variation in position in response to bright light quoting Fritsch (1935, P.214)*, and where each recorded distinct forms of chloroplast for various taxa within the same species, the records of the two authors did not coincide.

In view of the variation shown in the present study, Chapman and Bliding may simply have described arbitrary points on a range of variation of one "plastic chloroplast" type. The results cited above are insufficient to indicate how likely this is. Only two facts have been established:-

(1) The chloroplast of one South Island Enteromorpha population is very plastic.

(2) Modification of this chloroplast commenced very early in ontogeny.

Figures 36 and 37 show the basal and distal regions respectively of a plant only 262.5 μ long, in which many of the modifications described in the preceeding paragraphs are evident. It was not possible to draw any positive conclusions until other populations had been examined in the same way. Two additional 20 plant samples were therefore taken from a Motunau River population. Sample II (Table 10) represented an

---

* However, Fritsch (1935, P.214) did not state that the chloroplast in Enteromorpha shifted its position in response to bright light.

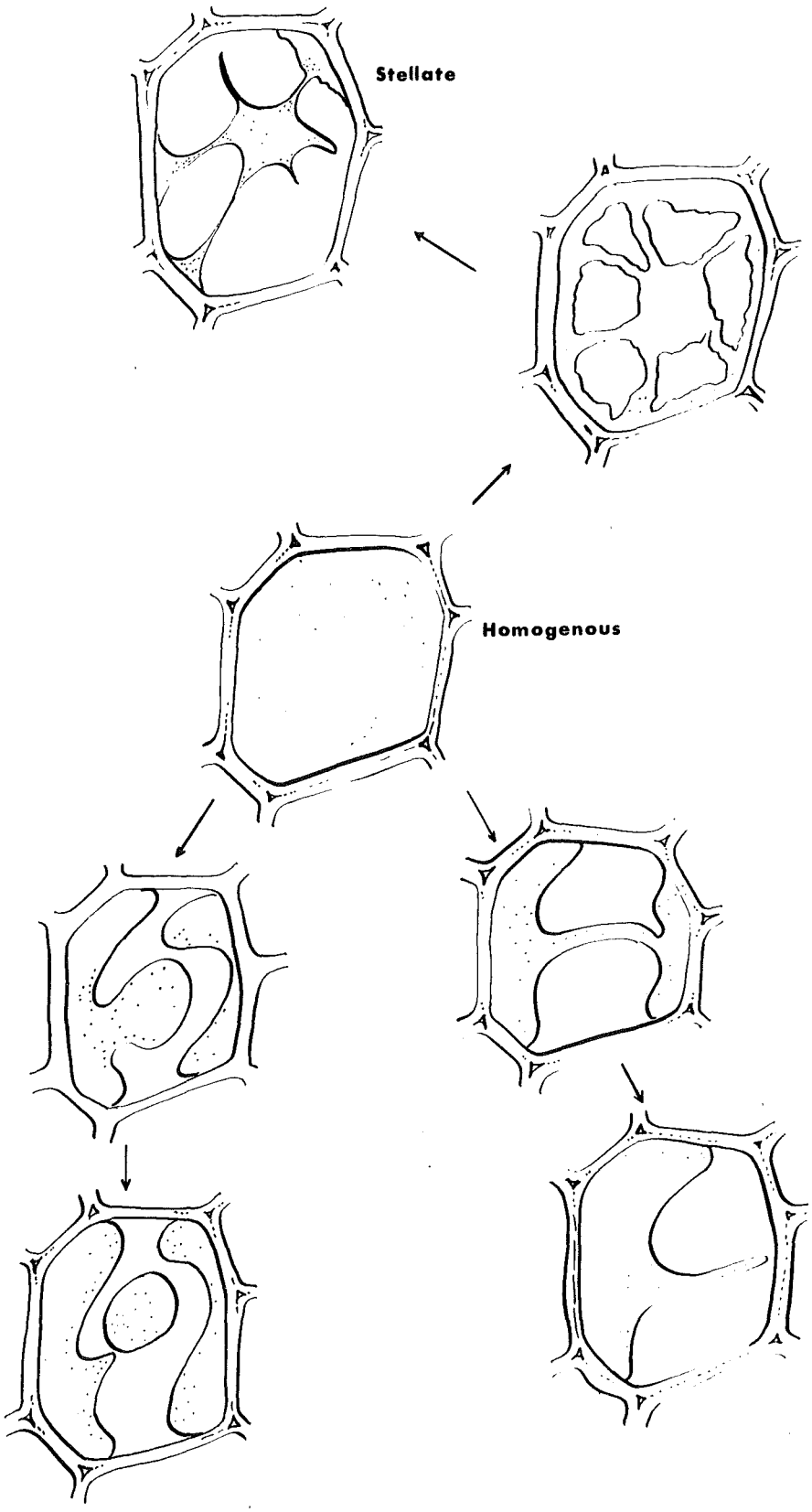


FIGURE 39 - Refer to text for explanation.

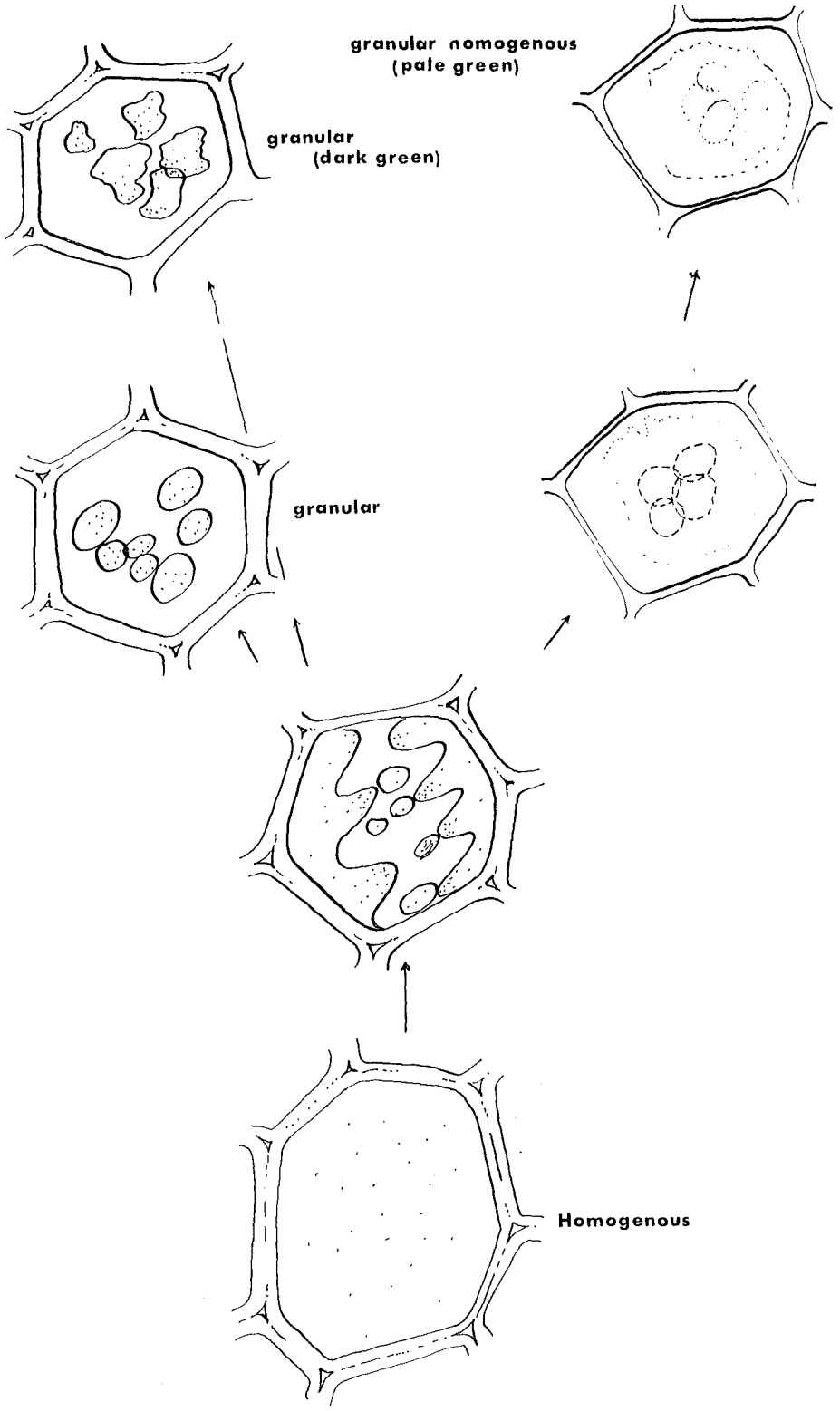
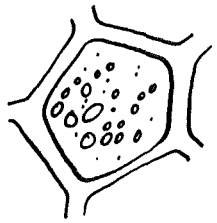
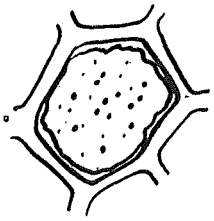


FIGURE 40 - Refer to text for explanation.

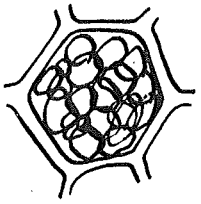
Types of chloroplast recognised in Enteromorpha Sample II (an early maturing stand of summer generation 1) from point c in the Motunau River. The chloroplast numbers used in the opposite table are shown down the left hand side of the page.

1. 

Chloroplast divided into numerous small granules of various sizes.

2. 

Chloroplast almost completely homogeneous rarely filling the cell.

3. 

The whole chloroplast divided into a number of irregular granules, all considerably larger than in (1) above, and darker in colour.

TABLE 10 A

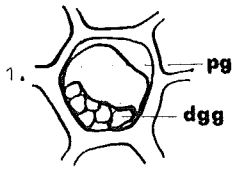
TABLE 10 - Results of Sample II. The Distribution of Chloroplast Types in a 20 Plant Sample of an Enteromorpha Population from the Motunau River.

Plant Number	Chloroplast type (the numbers are those from the facing table).		
	Basal Region	Median Region	Distal Region
1	1	2	3
2	2	2	1
3	2	2	2
4	2	2	1
5	1	2	2
6	2	1	3
7	2	1	3
8	2	3	1
9	1	3	1
10	2	3	3
11	1	3	3
12	2	2 (1)	3
13	2	3	3
14	2	2	2
15	2	3	3
16	2	2	2
17	2	2	3
18	2	2	3
19	2	3	3
20	2	2	3

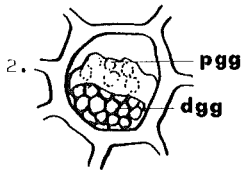
SUMMARY

Chloroplast Type	Basal Region	Median Region	Distal Region
1	4	2	4
2	16	11	4
3	-	7	12

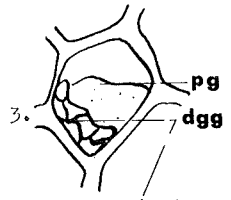
Types of chloroplast recognised in Enteromorpha sample III (a late maturing stand of summer generation 1) from point B in the Motunau River. The chloroplast numbers are shown down the left hand side of the table.



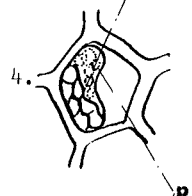
Small dark green granular region (dgg) appressed to one wall, with a larger pale green area (pg).



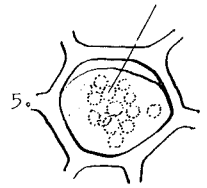
A more extensive dark green granular region (dgg) than in (1), and a pale green faintly granular (pgg) area occupying 75% of the lumen.



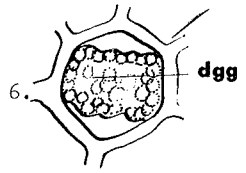
Small dark green granular (dgg) area with a pale green region (pg) occupying up to 75% of the lumen.



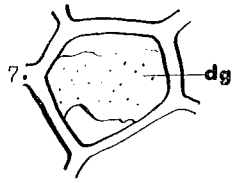
Small dark green granular area (dgg) with a pale green faintly granular region (pgg) present in some cells only, occupying 50% of the lumen.



The chloroplast consists solely of a pale green faintly granular region (pgg) occupying about 75% of the lumen.



Chloroplast consists solely of a dark green granular region (dgg) occupying from 60 - 80% of the lumen.



Dark green non granular chloroplast (dg), differing only from (6) above in the lack of granulation.

TABLE 11 A

TABLE 11 - Results of Sample III. The Distribution of Chloroplast Types in a 20 plant sample of an Enteromorpha population from the Motunau River.

Plant Number	Chloroplast type (numbers are those from the facing table).		
	Basal Region	Median Region	Distal Region
1	1	2	2
2	1	2	4
3	6	3	5
4	7	3	4
5	1	3	2
6	7	7	4
7	7	4	4
8	7	3	4
9	7	3	5
10	7	1	3
11	7	3	5
12	7	3	4
13	7	3	3
14	7	3	4
15	1	3	3
16	7	3	3
17	1	3	3
18	6	3	3
19	6	3	3
20	6	3	3

SUMMARY

Chloroplast Type	Basal Region	Median Region	Distal Region
1	5	1	-
2	-	2	2
3	-	15	8
4	-	1	7
5	-	-	3
6	4	-	-
7	11	1	-

early maturing stand of Summer Generation one*, sample III (Table 11) a late maturing stand of the same generation. Each was treated in the same manner as the first sample.

The types of chloroplast recognised in sample II appear in Table 10A, those in sample III appear in Table 11A.

The basal and median regions of sample II were dominated by a homogeneous type of chloroplast, the distal regions by a coarsely granular chloroplast. Most plants had an acropetal transition from homogeneous to coarsely granular plastids. All the recognised types occurred in the median and distal regions, two in the basal regions.

A similar condition was found in sample III, with a greater range of chloroplast type. The basal regions were dominated by a dark green homogeneous chloroplast. The median and distal regions by one with a small dark green granular and large pale green faintly granular area. However, other types were recognised in the three regions. These are shown in the summary of Table 11. The plastids in samples II and III were generally located in the distal portion of the cell, facing the apex of the thallus.

---

* During the summer new plants appeared to be established continually. However, a large number were established about December, and at maturity these remained morphologically distinct from those plants established earlier. The plants established at the beginning of the summer were termed Summer Generation one to distinguish them from the younger plants established later, referred to as Summer Generation two.

These three samples were considered sufficient basis for the conclusions that a range of variation is likely to be found in the chloroplasts, (1) of any South Island Enteromorpha population, (2) at any time during the growing season, (3) the total variation in any population is dependent on the inherent variability of its chloroplast, and (4) the length of time necessary for it to develop all possible forms.

The work described in the following paragraphs was carried out (1) to determine the total chloroplast variation in a sample population and (2) to follow the ontogeny of the various chloroplast forms. This was achieved by growing one population through several generations in laboratory culture, which accentuated the need for information on the influence of environmental factors on chloroplast morphology. Because a vast number of artificial environments could be created by various combinations of such factors as culture medium, pH, temperature and illumination, a combination closely resembling that in the natural environment had to be chosen.

The conditions under which the experimental population was to be grown. In the literature it was repeatedly indicated that the environment had a considerable effect on the chloroplast. Bliding (1944, 1963) showed that chromatophore size and colour could be altered by varying the nutrient supply and light intensity. Womersley (1956) attributed the extreme paleness of many South Australian Enteromorpha compressa plants to exposure to bright sunlight. Algeus (1951) demonstrated that the excess of short wave lengths in bright sunlight could cause the complete disappearance of algal pigmentation.

However, Gayral (1960) noted that the form of plastid in Ulva linearis, Enteromorpha flabellata, Blidingia and Rhizenteron did not alter up to a certain age regardless of the cultural conditions.

It was necessary to know precisely what modifications could be produced by varying the cultural conditions. If a variation could be produced by cultural conditions within the expected range of the natural environment, this would have to be taken into account in the total variation. Two experiments were established to determine whether chloroplast morphology could be artificially altered. Firstly, the influence of varying light intensity in a medium of normal sea water was studied, and secondly, the influence of high light intensity under slightly acidic but nutrient rich conditions. The cultural conditions most closely resembling those in the natural environment were selected by (1) comparing the range of natural chloroplast variation already recorded, with that produced artificially, and (2) by comparing the growth rate of plants in artificial culture with those in the natural environment.

The experimental modification of chloroplast form. Experiment I. The establishment of cultures to determine the effect of increasing light intensity in a medium of normal sea water. A number of small, fertile, unbranched plants of Summer Generation two from the Motunau River, were washed in autoclaved sea water and placed in a beaker of sterile sea water until zoospore release occurred. The zooids were then pipetted out onto microscope slides and covered with a long No. 1 cover slip, to retain the reproductive bodies together as much as possible. (Had plants any bigger than germlings been required, it

FIGURE 41 - Two sporelings grown in sterile sea water, illuminated by sunlight, filtered through six thicknesses of glass. x100

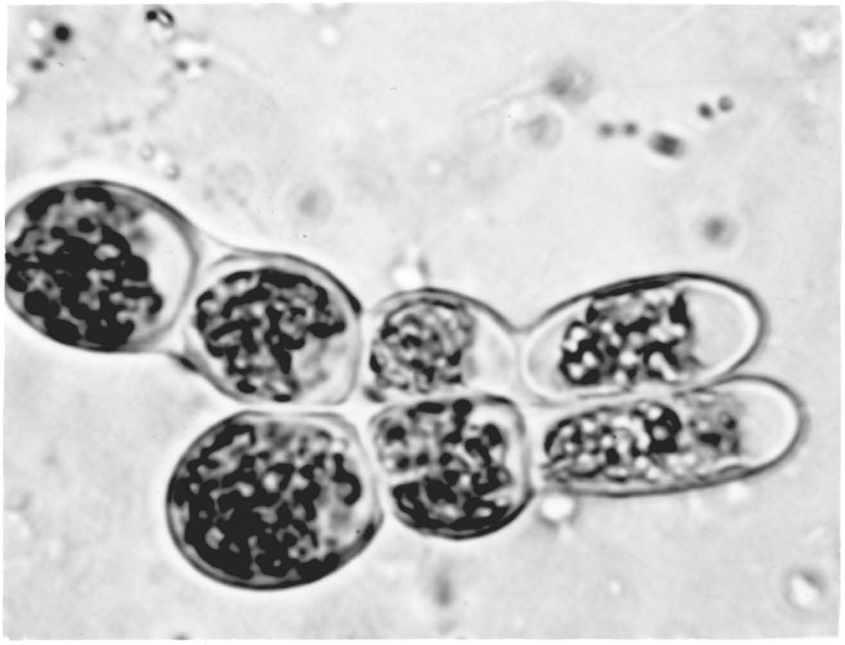


FIGURE 42 - The same two sporelings as in Figure 41, showing the finely granular chloroplasts. x600

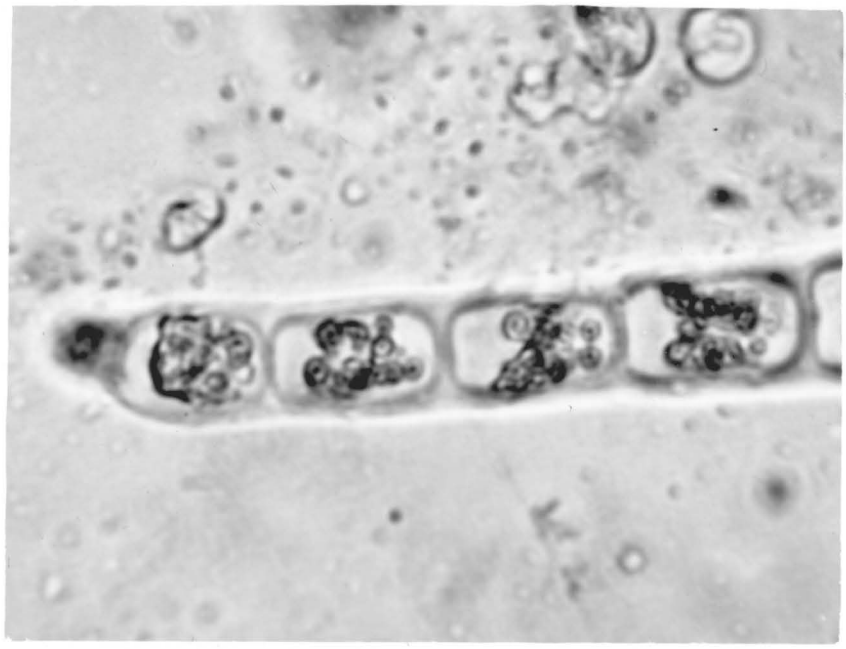


FIGURE 43 - The tip of a sporeling grown in sterile sea water and illuminated by sunlight filtered through four thicknesses of glass.

would obviously have not been practical to burden them with a cover slip.) Each was stood at a 45^{o} angle in a beaker of autoclaved sea water covered with a large watch glass.

The object of the following experiment was to duplicate the variation of conditions in the natural environment as closely as possible. Enteromorpha intestinalis grows at varying depths in the river. As water absorbs ultra violet light, plants growing in deep water would receive less intense light, containing a smaller quantity of u.v. wave lengths than those near the surface.

To simulate the decrease in intensity with increasing depth, the beakers containing the experimental plants were arranged in 3 rows at distances of 3 inches, 10 inches, and 24 inches away from the laboratory window. Those 3 inches away received a maximum intensity of 290 foot candles. U.v. light was absorbed in increasing amounts by shielding each row of vessels with 2 thicknesses of glass. In the following discussion the beakers 3 inches away from the window protected by two additional thicknesses of glass (T2) are represented by 3" T2 the second series, 10" away 10" T4, and the third series 24" T6. From 3" T2 - 24" T6 the plants would be subjected to light of decreasing intensity and varying quality.

The establishment of cultures to determine the effect of high light intensity on plants in a nutrient rich acidic culture medium.

Experiment II. The cultures were set up in the same way as Experiment I, but maintained in a modified culture medium. To every 100 cc of autoclaved sea water was added .2% Na No.$_3$ .04% $Na_2 HPO_4$ and two standard spoonsfull (.092g) of Carbonate buffer (as per D.E.G. Sheat's

19 Ph.D. thesis). The initial pH was 6.9. The cultures were protected from the direct rays of the sun by only two thicknesses of glass.

Results. Experiment I. This series of cultures maintained in sterile sea water protected from the sun's rays by 2, 4, and 6 thicknesses of glass, showed 3 lines of modification with decreasing light intensity and smaller quantities of u.v. wave lengths.

The chloroplast developed (1) deeper green coloration, (2) finer granulation, and (3) occupied an increasing proportion of the lumen.

The plants most protected from bright sunlight (24" T6) had dark green chloroplasts which appeared almost homogeneous under low power (Figure 41), finely granular under high (Figure 42), and filled 90-100% of the lumen. Those protected by 4 thicknesses (10" T4) had more granular chloroplasts filling about 50% of the lumen (Figure 43). The plants protected by only 2 (3" T2) thicknesses of glass had paler green chloroplasts composed of a few large granules filling about 40% of the lumen (Figure 44).

These results are in accordance with those of Bliding (1944) but at variance with Gayral (1960).

Results. Experiment II. The plants maintained under acidic nutrient rich conditions caused a natural pH swing from 6.9 - 7.2 during their 6 weeks growth, and the chloroplasts were greatly modified compared with those in the preceeding experiment.

In contrast to the plastids referred to by Algeus (1951) these did not completely disappear. Instead, they formed a narrow pale green

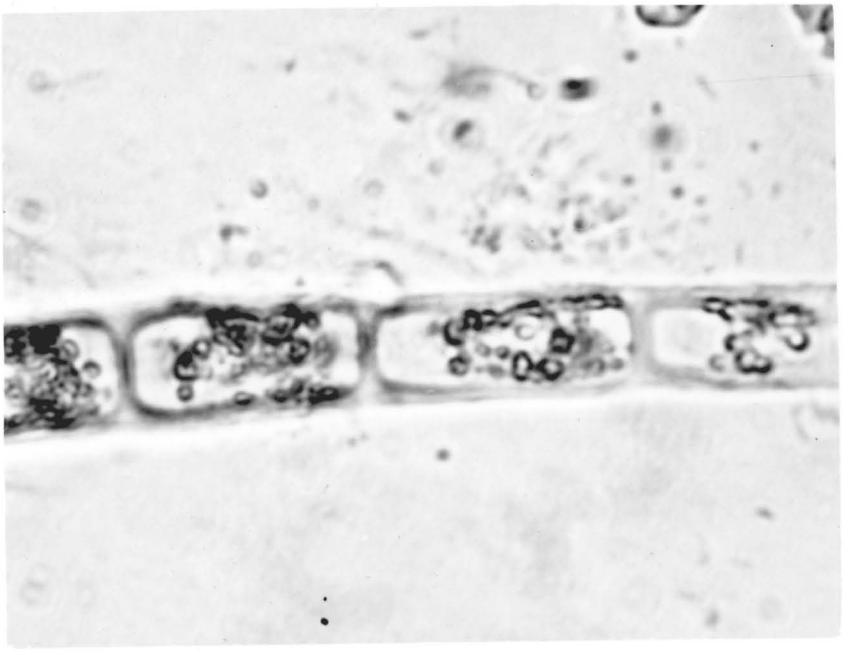


FIGURE 44 - Median section of a sporeling grown in sterile sea water and illuminated by sunlight filtered through two thicknesses of glass.

FIGURE 45 - The distal region of a plant grown under mildly acidic nutrient rich conditions and illuminated by sunlight filtered through two thicknesses of glass. The morphology of the chloroplast has changed completely, it now forms a narrow parietal band of green about three walls. These plants were difficult to photograph. The outline of one chloroplast has been inked in to assist interpretation.

homogeneous band attached to 3 walls, but they certainly were difficult to see at first. Granular inclusions, possibly pyrenoids, about 1.9μ in diameter, were present in each cell, along with a number of smaller inclusions (Figure 45). From this experiment it was not completely clear whether the bright sunlight or acid conditions caused the drastic modification of the chloroplasts. The experiment was therefore repeated with the pH adjusted with carbonate buffer so that a natural swing from 7.72 - 8.94 occurred in 8 weeks. This produced chloroplasts comparable with those of plants grown in sterile sea water behind 4 thicknesses of glass. However, the two cultures are not directly comparable, as the growth rate was considerably faster under the more alkaline conditions, and some mutual shading of plants also occurred.

From these experiments the following conclusions regarding chloroplast morphology may be drawn. Increasing exposure to strong sunlight causes the chloroplasts (1) to occupy less of the lumen, (2) to become a paler green, and (3) to develop larger granules. There is no evidence that chloroplasts disappear when growing healthily in bright sunlight. However, (4) under slightly acidic conditions the chloroplast becomes particularly pale in colour and, (5) loses all indications of granulation, but the pyrenoids appear to remain.

The growth rates and chloroplast morphology of the plants grown in culture were compared with those in the natural environment. Those conditions which most closely approached the latter were:-

culture medium: Autoclaved sea water with no pH adjustment.

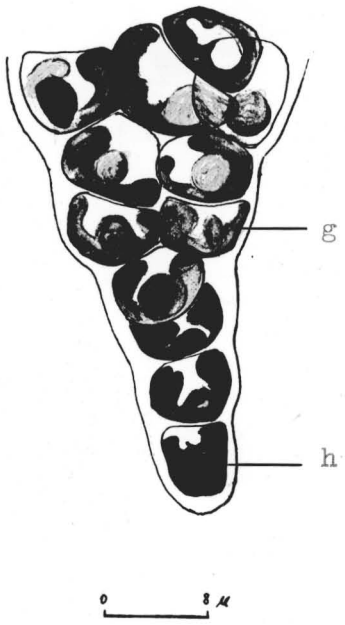


FIGURE 46 - The distal region of an immature winter generation plant raised in culture. Two types of chloroplast are shown, homogeneous (h) and a developmental stage of the coarse granular chloroplast (g).

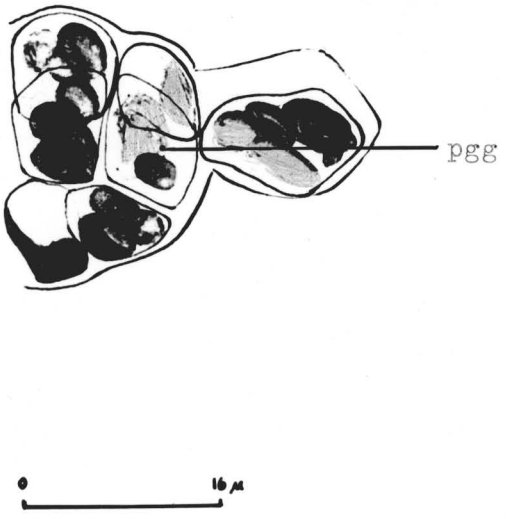


FIGURE 47 - The distal region of a mature winter generation plant raised in culture, showing one of the mature forms of chloroplast - pale green granular (pgg).

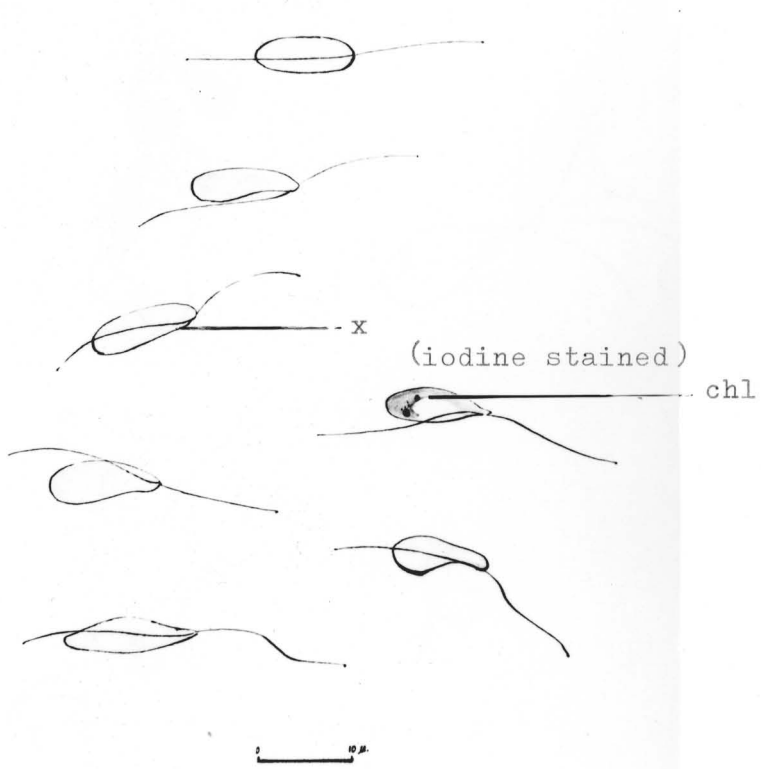


FIGURE 48 - Gametes from the winter generation Motunau. (chl) chloroplast, (x) appearance of unstained gametes.

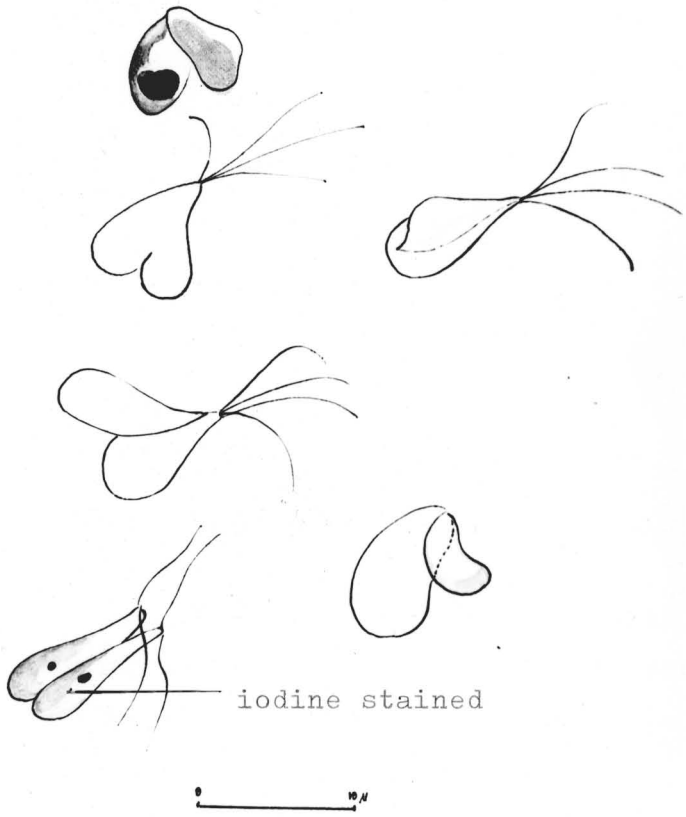


FIGURE 49 - Gametes from the winter generation at Motunau during the initial stages of copulation. x600

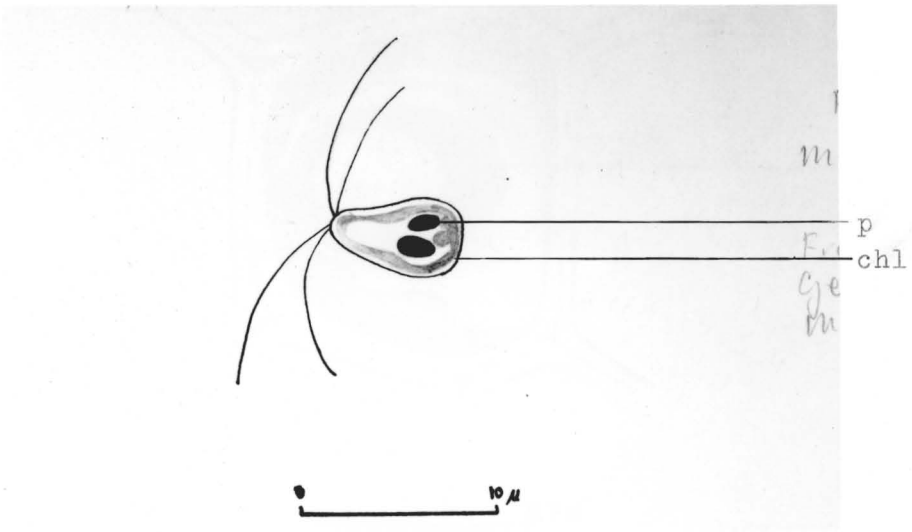


FIGURE 50 - A planozygote from the winter generation Motunau, showing chloroplast (chl) and two prominent pyrenoids (p).

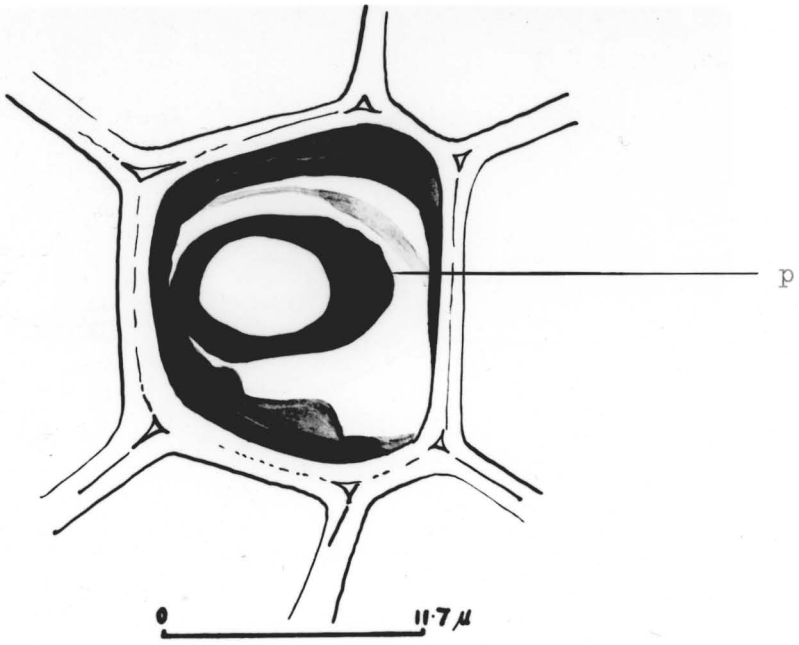


FIGURE 51 - The cell of a summer generation plant raised in culture, showing chloroplast type 1. The pyrenoid (p) is also shown.

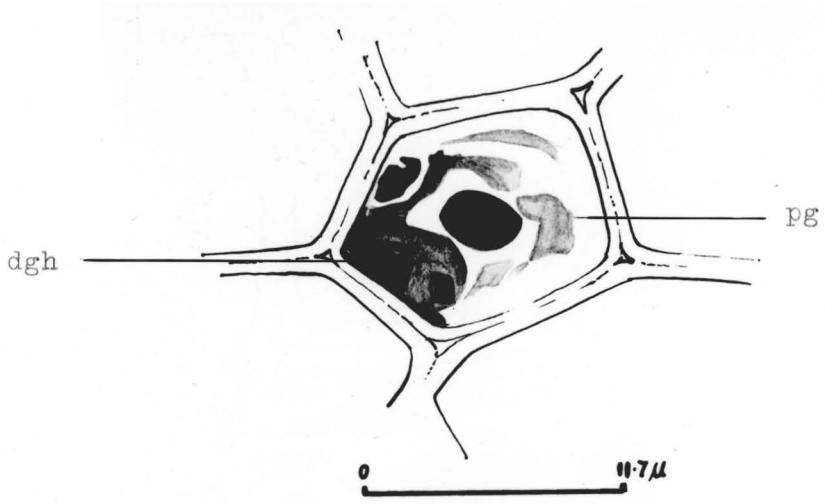


FIGURE 52 - The cell of a summer generation plant raised in culture, showing an intermediate stage in the formation of chloroplast type 2. The remainder of the homogeneous dark green region (dgh) and developing pale green granules (pg) are also visible.

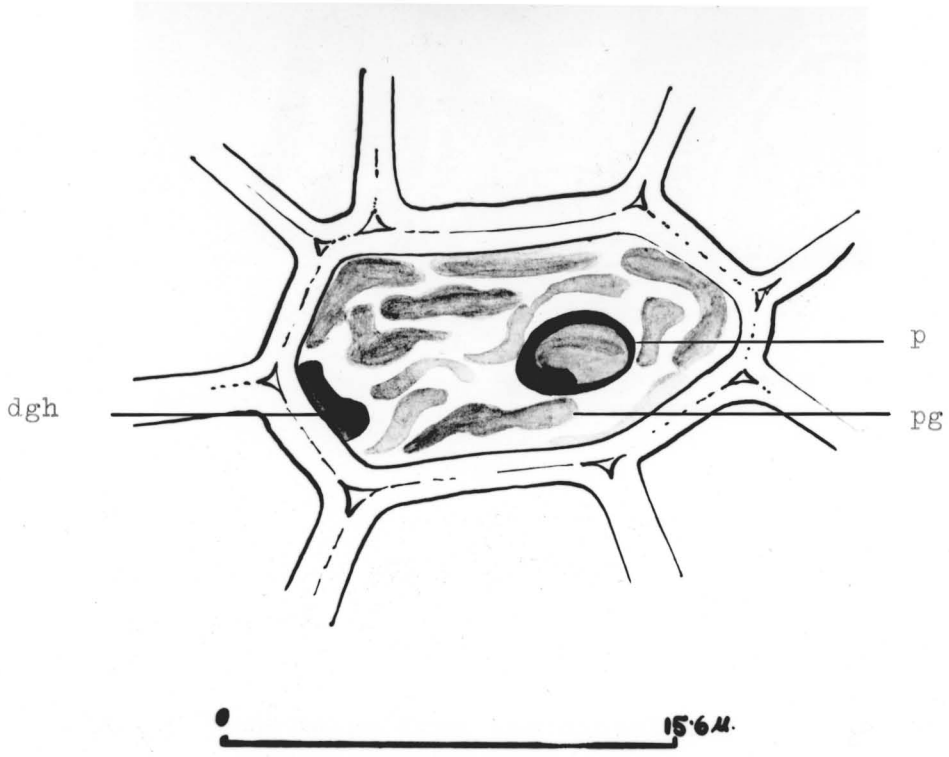


FIGURE 53 - The cell of a summer generation plant raised in culture, showing pale green granules (pg), the remainder of the dark green homogeneous chloroplast (dgh) and a single pyrenoid (p). Chloroplast type 2.

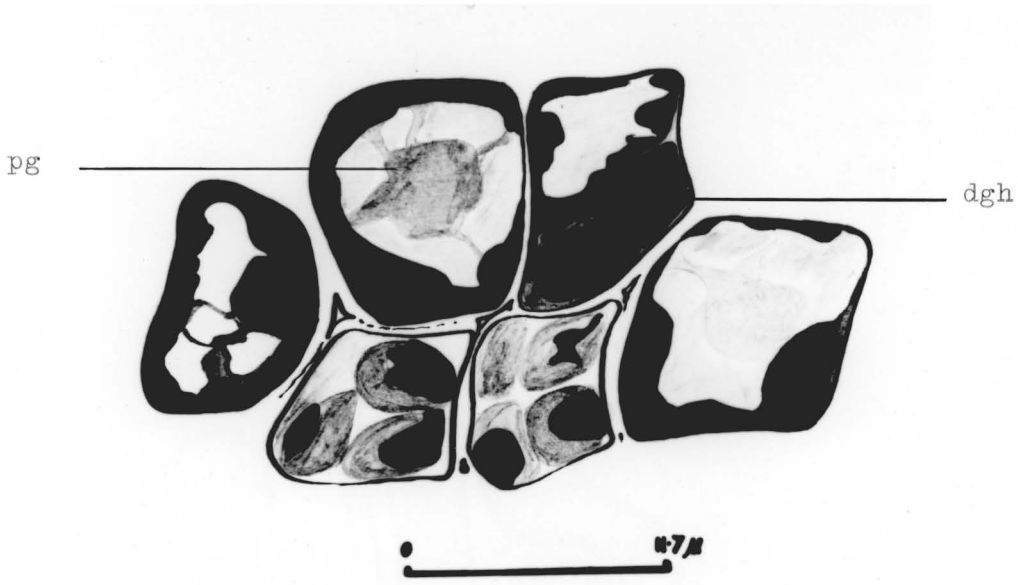


FIGURE 54 - Cells from the distal region of a Summer Generation Plant raised in culture. The development of localised pale green areas (pg) from a homogeneous dark green chloroplast (dg) is shown. Zoospores with both pale and dark green regions of plastid, and pale green regions alone, are shown.

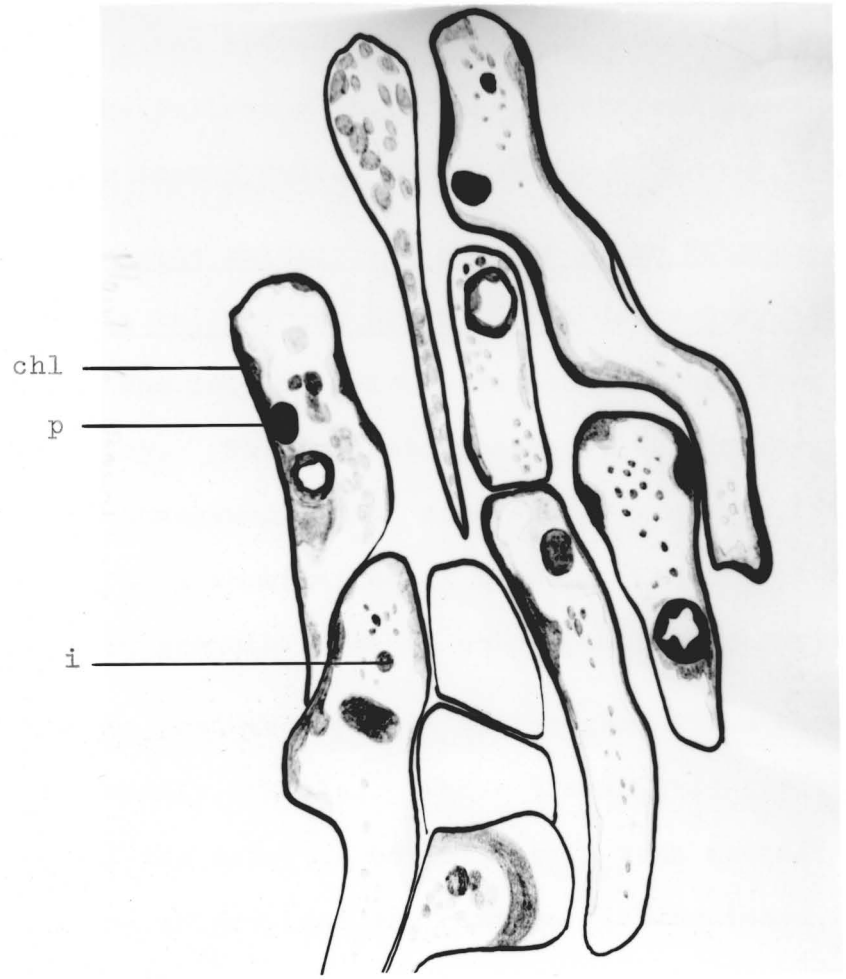


FIGURE 55 - Cells from the stipe region of an Enteromorpha plant raised in culture, showing chloroplast morphology. The chloroplast (chl) is distributed along one or two walls. The pyrenoid (p) remains prominent, and the lumen contains a number of inclusions (i) which respond positively to iodine. These could be small pyrenoids or granules of stromatic starch.

(Contrary to Droop's (1960) statement, autoclaving did not cause a significant Ca Co_3 precipitation and acid pH swing.) Only enough water was added to compensate for evaporation losses.

Illumination: Sunlight from a north window filtered through 6 thicknesses of glass spaced over 2 feet of bench. The cultures required for the following study were maintained under these conditions, some for longer than a year.

The ontogenetic chloroplast changes of an Enteromorpha population originating from the Motunau River, North Canterbury, South Island, New Zealand. The initial culture was established from gametes of the winter generation. These plants possessed the following types of chloroplast:- homogeneous with some coarse granules (Figure 46), fine granules, or a combination of these. The distal regions usually had a pale green granular type of chloroplast at maturity (Figure 47).

The gametes produced by this winter generation have a chloroplast which is not readily visible. Under transmitted light they appear pale blue. If the water is coloured by a weak iodine solution to act as a filter, the chloroplast may just be distinguished, (Figure 48).

It is not naturally visible until late stages of gamete fusion. Figure 49 shows gametes during copulation, Figure 50 a motile zygote with a visible chloroplast.

The mature plants into which these zygotes grow are dominated in all regions by a homogeneous dark green chloroplast with a large pyrenoid (Chloroplast type 1, Figure 51). Modification may occur to this chloroplast anywhere in the thallus, but more frequently in the fertile regions.

The whole chloroplast, or a portion of variable size may become pale green. Fragmentation usually accompanies a colour change affecting the whole chloroplast. Figure 52 shows an intermediate stage in the formation of a pale green granular chloroplast and Figure 53 a mature chloroplast of this type (chloroplast type 2). More often, only a portion of the homogeneous chloroplast becomes pale green, and this is frequently located in the centre of the lumen (P.G. Figure 54). As a result the zoospores possess a combination of light and dark green areas of chloroplast, or one type alone. Zoospores with two types are shown in Figure 54.

The majority of the dark green homogeneous chloroplasts develop some pale green regions before the cells become reproductive. As a result the whole thallus changes from dark green at the base, to olive green in the fertile region.

In the stipe all cells undergo a colour change - from dark to pale green. During rhizoid formation the chloroplast becomes distributed along one or two walls as a narrow band. The pyrenoids are then the most prominent structures, and the lumen generally contains a number of small irregular bodies which also respond positively to iodine. These could therefore be small pyrenoids or granules of stromatic starch, labelled inclusions in Figure 55.

The zoospores produced by this summer generation may possess a pale green homogeneous chloroplast or one with both dark and pale green regions (Figure 56). The dark green is however lighter by comparison with the basal region of the vegetative thallus.

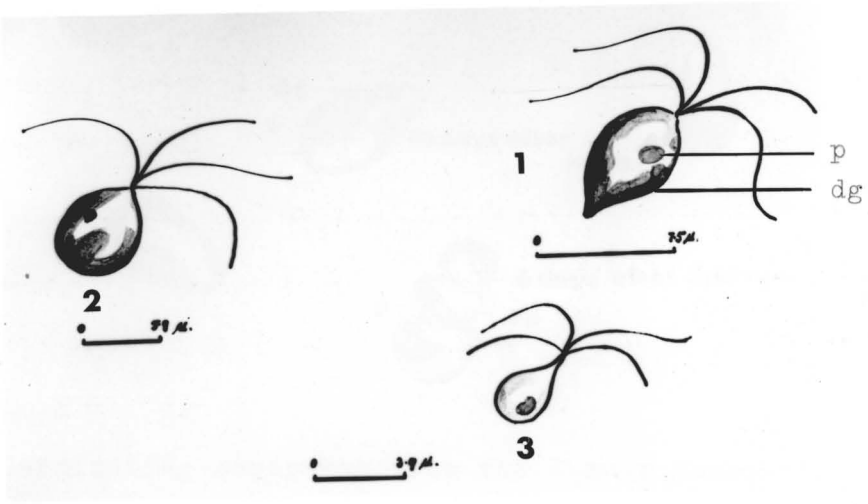


FIGURE 56 - Zoospores from the Summer Generation of Enteromorpha raised in culture.

(1) a zoospore with both dark green (dg) and pale green regions of chloroplast, pyrenoid (p),

(2) a zoospore containing only a dark green chloroplast,

and (3) one containing only a pale green chloroplast.

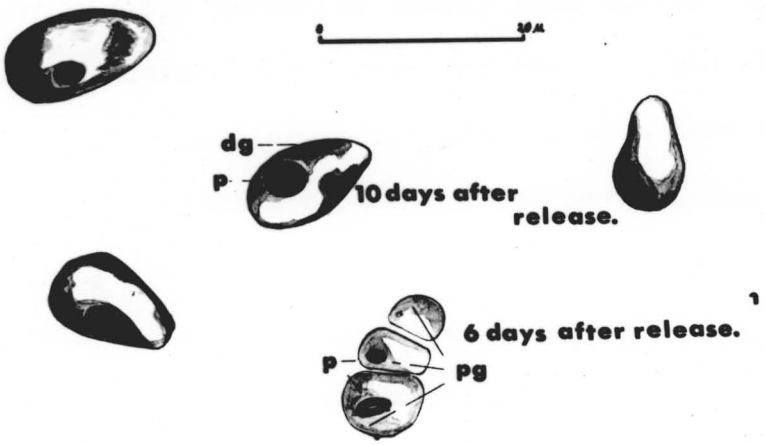
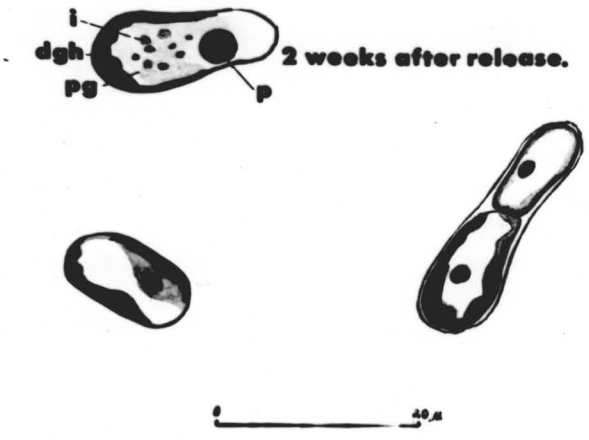


FIGURE 57 - Germinating zoospores from the Summer Generation Motunau grown in culture. The various ages of the sporelings are indicated above. DGH dark green homogeneous portion of the chloroplast. PG pale green portion. i. inclusions, which could be small pyrenoids or stromatic starch. P pyrenoids.



Following settlement all zoospores pass through the following stages. During the first week all chloroplasts become pale green and homogeneous. Few have visible pyrenoids during this stage. After about 10 days the plastid becomes dark green and the pyrenoid reappears and / or undergoes an increase in size, corresponding with that of the cell. Cell growth continues during the 10 - 14 day period, but that of the plastid ceases. Instead, a region appears to become firmly attached to the cell wall and as a result of cell expansion, dissociated from the main body of the chloroplast. This separated portion turns pale green (P.G. Figure 57), its change in colour caused either by its extreme thinness, or a localised physiological change.

Once the multicellular stage is reached, the homogeneous dark green chloroplast (characteristic of 10 days of growth, Figure 57) becomes restricted to cells in apical or other very actively dividing regions. Cells possessing varying pale green areas, some separated into granules, are characteristic of slower dividing areas of the thallus.

The chloroplasts in the Motunau River population therefore undergo a cyclic change in morphology and colour, in each generation. These may be considered at two levels:

(1) that of the individual cell,

and (2) the level of the whole thallus.

Chloroplast changes in the individual cell are of three types:

(1) During the unicellular to multicellular stage of plant development, the chloroplast passes through homogeneous pale green and homogeneous dark green stages to a mature form of morphology with various types of pale green area.

(2) When the plant has progressed beyond this stage of ontogeny, the homogeneous dark green chloroplast normally becomes restricted to actively dividing apical regions. Cells produced there undergo a change from a homogeneous dark green to a mature form of chloroplast. Actively dividing cells occur less often in intercalary regions, where their presence is again associated with this chloroplast change.

(3) Slower dividing intercalary cells possess mature forms of chloroplast and it seems likely that they only pass this form of chloroplast on.

The changes in colour and morphology at the thallus level are as follows: The immature stage of thallus development is that in which it is entirely dark green, and the apical region is associated with active cell division and homogeneous chloroplasts. The slower dividing intercalary regions then become reproductive, causing the loss of the apical region (Ramanathan, 1939). The mature thallus thereby produced has a transition from a dark green thallus base to a distal pale green fertile area.

These changes are cyclic, occurring in each generation.

In all likelihood, the factor(s) which causes the colour change in individual areas of the chloroplast also causes the colour change in the whole thallus. In addition, there are several degrees of thallus colour change. It is therefore of importance for the taxonomy of the group, to determine the nature of these factors. A fuller discussion of this is therefore included in the following section.

The total range of 4 chloroplast types for this summer generation is considerably less than that occurring in the natural environment. This is not unexpected in view of the chloroplast plasticity, the influence of the environment upon it, and the constancy of the cultural conditions. The latter may also explain the comparatively small range found by Bliding (1944).

Summary and Conclusions from the sections dealing with:-

(1) the natural variation of chloroplast morphology in a population at any time during the growing season,

(2) the experimental modification of chloroplast form,

and (3) the ontogenetic chloroplast changes in a single population.

Summary. A range of chloroplast morphology is frequently found in South Island Enteromorpha populations. It can be experimentally demonstrated that the chloroplast is an extremely plastic organelle. E.g. moderate increases in light intensity cause it to occupy less of the lumen, in agreement with Bliding's (1944) observations, while a great increase under slightly acidic conditions causes a complete morphological change. Algeus (1951) found that an excess of short wave lengths caused the complete disappearance of algal pigmentation. The high proportion of these waves could have caused the present morphological change. However, the writer feels that the results of Algeus apply only under certain conditions.

From the results of this investigation it appears that the greater the fluctuation of natural environmental conditions the greater is the variety of chloroplast form. Under cultural conditions closely duplicating those of the natural environment, it is possible to trace an

ontogenetic sequence between forms of chloroplast, recognised by several authors as separate distinct entities. The homogeneous type, from which all other forms appear to originate, is closely associated with a high rate of cell division.

Conclusions. In the section of this thesis dealing with cell diameter, it was established that the rate of division varied at random between regions of the one plant, and between comparable regions in different plants. At least a few homogeneous chloroplasts were found in most regions of all plants examined. Their presence therefore seems to be due to the random distribution of rapidly dividing cells throughout the thallus. This constitutes a correlation between chloroplast morphology and rate of cell division.

The existence of an ontogenetic cycle of change in morphology and colour as such does not appear to have been previously recorded. In view of this some reinterpretation of existing work is necessary.

(1) It is possible to reconcile the somewhat divergent records of Bliding (1944, 1955, 1960, 1963) with those of Chapman (1956, 1961). The present study has shown, that a range of chloroplast morphology is likely to be found in any Enteromorpha population; that this was smaller in cultured populations, and that the cultural conditions could radically alter the normal chloroplast morphology. Plants grown in sterile sea water illuminated by light filtered through 6 thicknesses of glass most closely resembled those from the natural environment.

Bliding described the chloroplast of plants grown in culture, Chapman those from the natural environment. Both may

simply have 'selected' a 'dominant' type to describe for each species, and if so they have frequently selected different forms for the same species e.g. Enteromorpha clathrata. It is possible that they described different points on a range of variation common to all races of this species. Bliding's particular cultural conditions may well have favoured only the disc shaped end of this range, Chapman's (natural conditions) the other, granular end. It is certainly not claimed that this is the first study to show any variation in chloroplast morphology. To the best of the writer's knowledge, this is the first study to show a pattern of relationship between the variants described in the literature.

(2) Because of the natural range of chloroplast variation, it is unwise to differentiate between forms closely resembling one another (as Bliding (1963) has done) by separate terms. It could be argued on the present evidence alone that these are not differentiae but synonyms.

(3) The writer would disagree with those definitions of the family Ulvaceae (Smith, 1938), Genus Enteromorpha (Fritsch, 1935; Taylor, 1960) and lesser taxa (Bliding, 1963; Chapman, 1956, 1961) which include one or two chloroplast types. Even if mention is made of the variability of some taxonomic characters, this provides no useful insight into the complexity of chloroplast variation.

Here it may be appropriate to point out the analogy between the taxonomic problem shown to exist by the results of this study and the situation in several groups of Angiosperms. The chloroplast in Enteromorpha may be considered heteroblastic sensu (Goebel, 1879; Davis and Heywood, 1963). The juvenile form is the homogeneous chloroplast, the adult form all those types derived ontogenetically from it.

In Enteromorpha, heteroblastism involves a cell organelle, in higher plants an organ or whole plant.

Nevertheless, the successful utilization of a heteroblastic character in any taxonomic system involves the same principle, one differing only in degree from that pertaining to more stable characters. This principle is the recording of the complete range of variation but for heteroblastic characters these records must be particularly thorough.

It is felt that if other populations were examined in the light of the results of this study, chloroplast morphology would become a useful taxonomic criterion. It is obvious why existing records were of little use in the classification of populations in the present study.

Chloroplast Colour and its Relationship to Thallus Colour. Two types of chloroplast colour change may be distinguished, (1) in the mature chloroplast individual areas frequently change colour, (2) at reproductive maturity the distal portion of the whole thallus is generally a shade paler than the basal region. Here the entire chloroplast of every cell has undergone a change.

It is important to determine the causes of these changes, as several taxa have been established almost solely on this basis, and subsequently criticised by several authors. If a pale green plant consistently gives rise to pale green plants under a variety of environmental conditions, and dark green plants behave similarly, there can be no doubting the validity of the character. The object of this section of the thesis was (1) to determine the causes of chloroplast colour changes and hence (2) to establish how much reliability could be placed on thallus colour as a taxonomic criterion.

Literature. J. Agardh (1883) separated Enteromorpha chlorotica with a pale green thallus, from E. compressa, solely on the basis of colour. Setchell and Gardner (1920) subdivided the Enteromorpha clathrata group of species on the basis of thallus colour and structure of the branch tips. Thus Enteromorpha crinata and E. erecta had dark green thalli in which the chromatophores filled the lumen, while E. plumosa and E. clathrata had pale green thalli in which they occupied less of the lumen. Chapman (1956, P.406) separated E. compressa with a dull to bright green thallus, from the various forms of E. procera, with light or pale green thalli, almost solely on the basis of colour. Within the assemblage of pale forms (E. procera) Chapman (1956, P.456)

separated E. procera f chlorotica from the others solely by 'the extreme paleness of the plants'.

Womersley (1956) considered that colour alone was insufficient to justify Agardh's 1883 separation of E. chlorotica from E. compressa. He states that 'such bleached drift specimens of E. compressa are of frequent occurrence'. Bliding (1944) demonstrated that thallus colour was an unreliable taxonomic criterion. Light intensity and nutrient availability greatly influenced the size of the chromatophore and therefore thallus colour. This was one of his bases for disagreeing with Setchell and Gardner's (1920) treatment of the E. clathrata species.

Field Observations. Observation of the range in thallus colour in a Summer Generation of Enteromorpha growing in the Motunau River suggested the following:- (1) that there were two distinct thallus colours, light and dark green (Figure 58), (2) both types exhibited a paler colour in the distal region (Figure 59). This was perfectly in accordance with the observations in the previous section, except for the fact that many of these plants were not fertile.

Further investigations revealed that the dark green plants grew in positions where they were probably exposed to direct sunlight for only very short periods. If Womersley's (1956) observation was correct it should be possible to induce dark green plants to become completely light green by exposing them uniformly to a high light intensity. By so doing, it should also be possible to determine whether this colour change is due to (a) a change in chloroplast morphology, or (b) a chemical change independent of form affecting the whole chloroplast. This was the objective of the following experiment.

FIGURE 58 - Left hand dark green, and right hand pale green plants belong to lower littoral and mid littoral zones respectively. Both have become convolute in spite of the great temperature difference between the two zones.

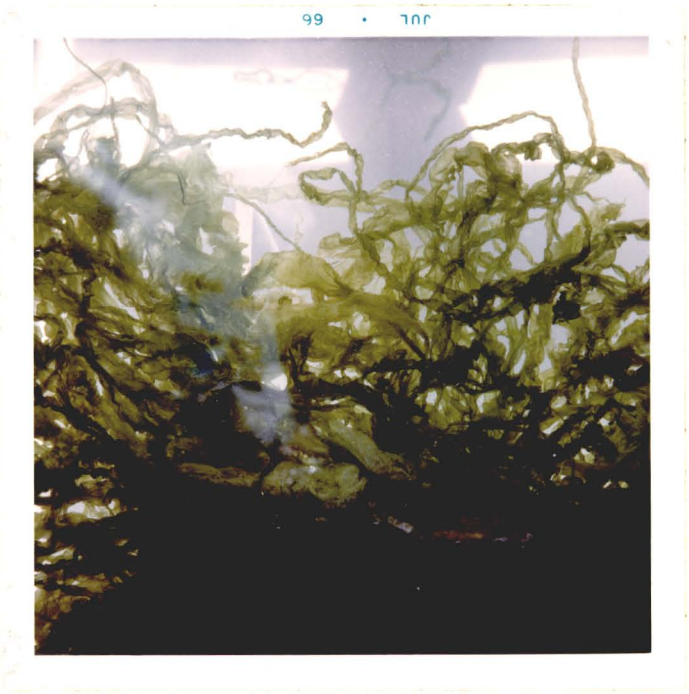


FIGURE 59 - Enteromorpha plants from the Motunau River, showing the gradation in colour between the top and bottom of the thallus.

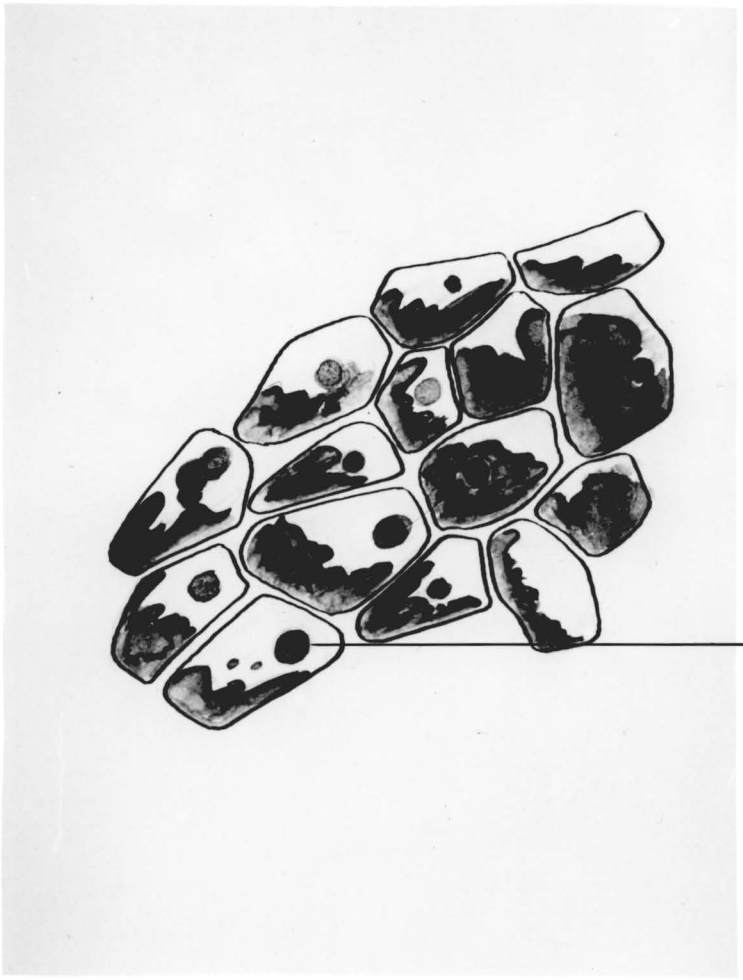


FIGURE 60 - Summer Generation 1: Control Cells of the basal region. (p) pyrenoid.

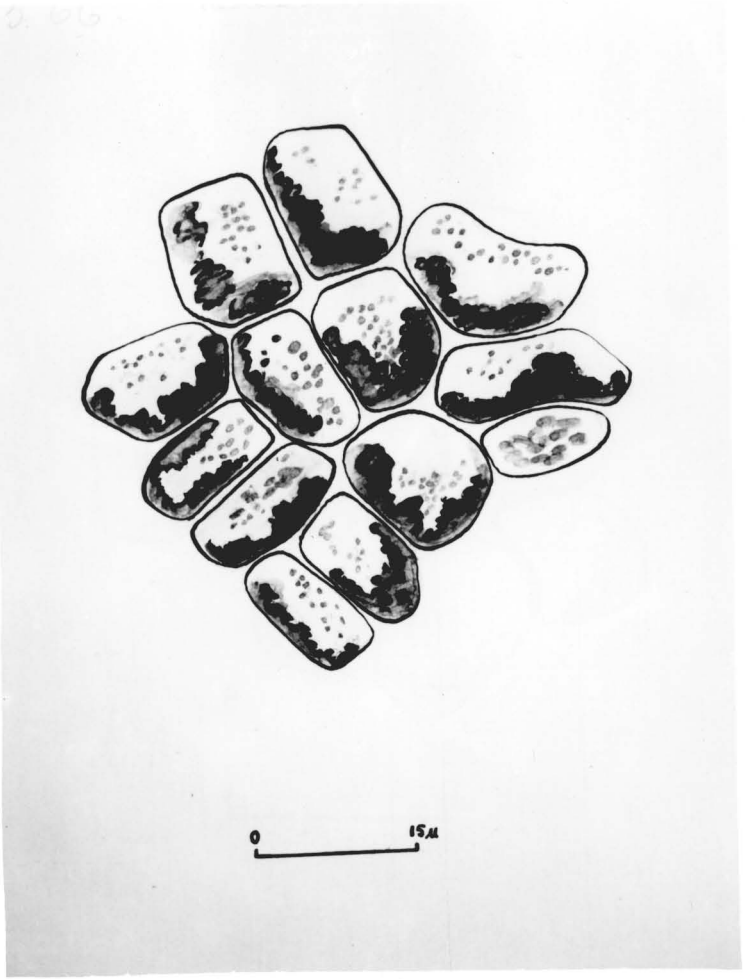


FIGURE 61 - Summer Generation 1: Control Cells of the Median region.

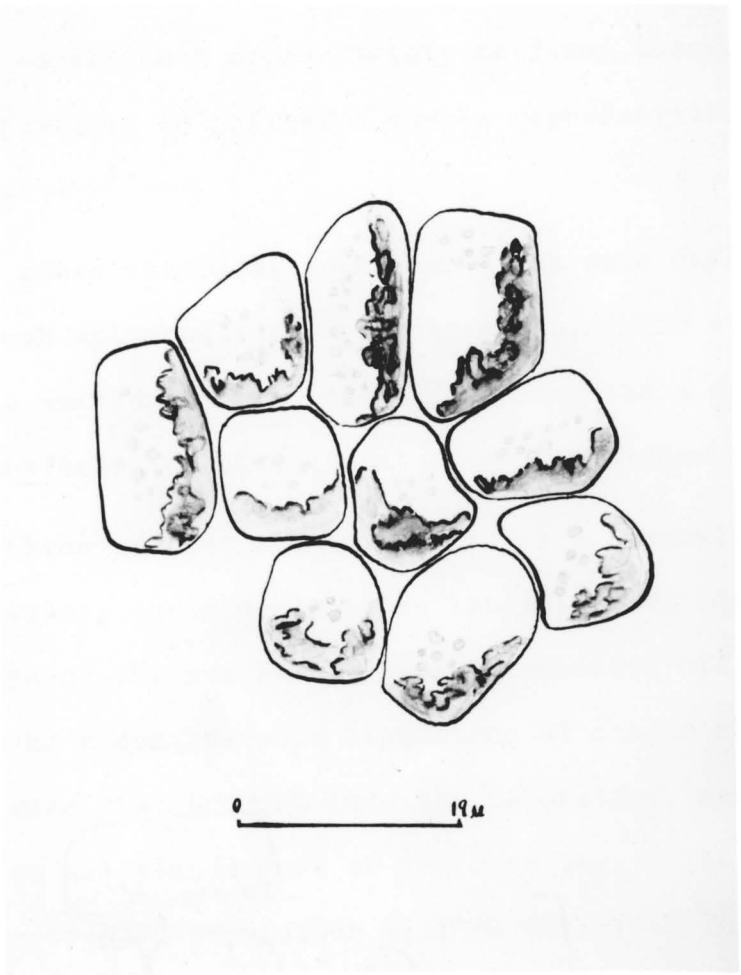


FIGURE 62 - Summer Generation 1: Control Cells of the Distal region.

Method. By following the pattern of colonisation in the Motunau River during the summer of 1965-66 at point A, it was possible to identify plants of Summer Generation one, namely plants established at the beginning of the season, and plants of Summer Generation two, namely plants established approximately half-way through the season. This made it possible to collect a sample representative of the whole summer growth.

Two dark green stands of each generation were selected for this experiment, each attached to a separate stone. One stand of each generation was marked and remained in the river as a control, the other was transferred to the laboratory in Christchurch.

Each of these samples was maintained in a separate vessel of filtered sea water, and placed in the laboratory window, shielded from the direct rays of the sun by only two thicknesses of glass. At the end of six weeks a considerable lightening of colour had occurred. The controls were then brought into the laboratory, and the following observations on all stands made on the same day. The control and the changes in the treated population will be described for each generation separately.

Chloroplast morphology. Control plants: Summer Generation 1. Both controls remained undisturbed in the natural environment during the 6 week duration of the experiment. The chloroplast morphology of basal, median, and distal regions of the summer generation 1 control is shown in Figures 60, 61, and 62 respectively. The chloroplasts in the basal region were homogeneous with faint indications of large granules. The plastids shrank progressively through the median and

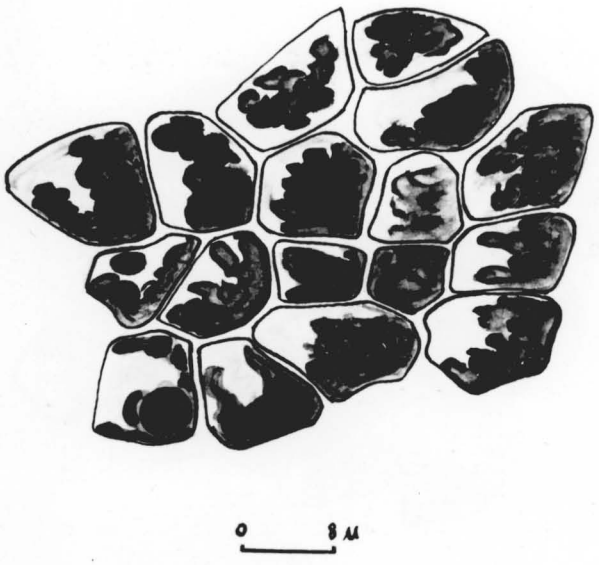


FIGURE 63 - Summer Generation 2: Control Cells of the Basal region.

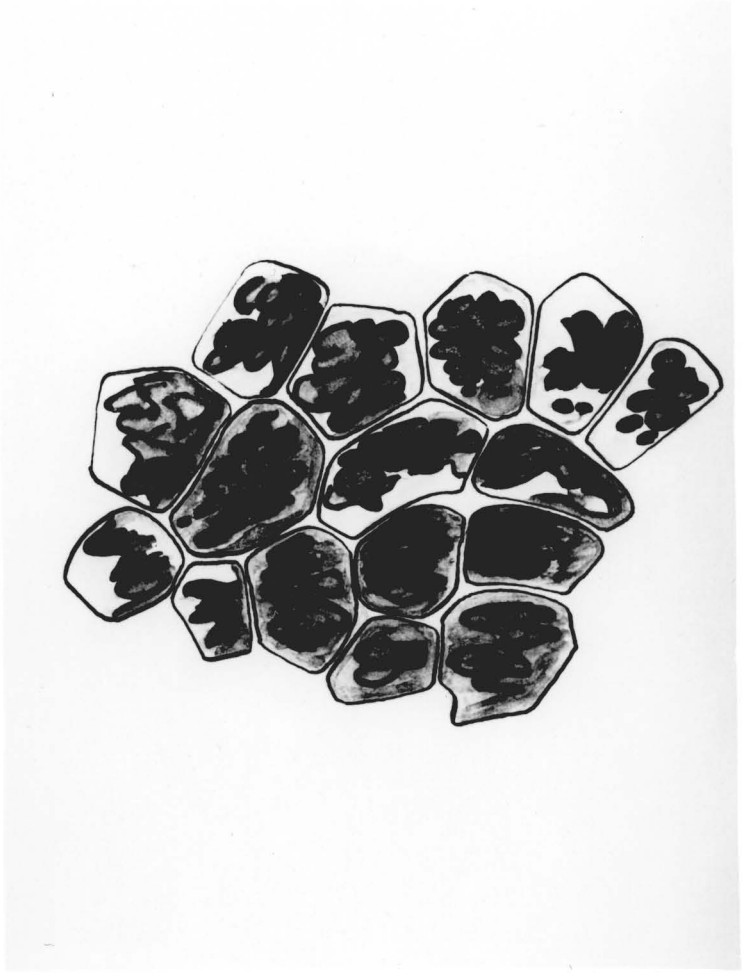


FIGURE 64 - Summer Generation 2: Control Cells of the Median region.

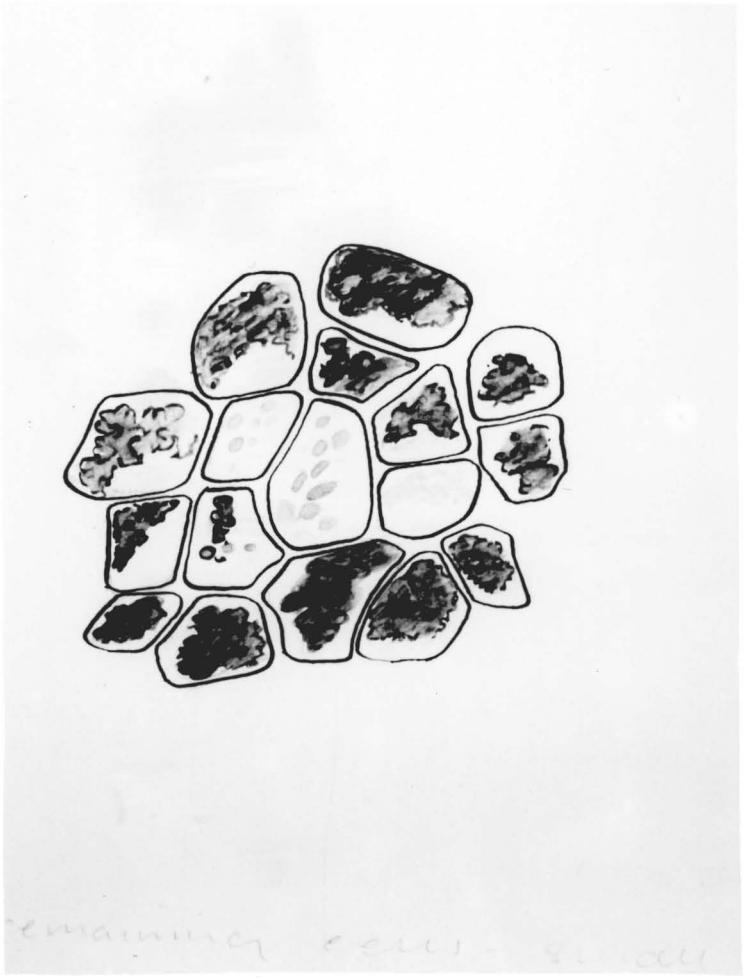


FIGURE 65 - Summer Generation 2: Control Cells of the Distal region.

FIGURE 66 - Plants of Summer Generation 1 from the Motunau River, after six weeks in bright sunlight at the laboratory window. Note the comparatively small change in colour, compared with the right hand specimen in Figure 58, or Figure 59 which closely resembled that of the control plants.

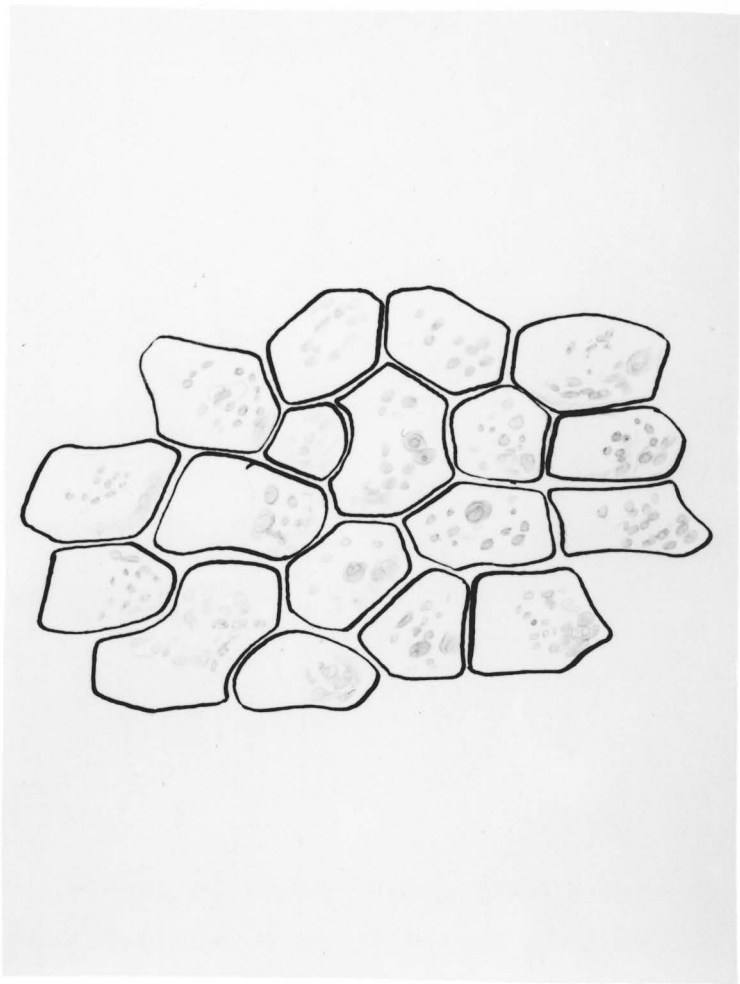


FIGURE 67 - Summer Generation 1. Cells of the Distal region after exposure to six weeks bright sunlight.

FIGURE 68 - Plants of Summer Generation 2 from the Motunau River, after six weeks in bright sunlight at the laboratory window. Note the relatively great difference between the colour of these plants and the left hand plant in Figure 58, which closely resembles that of the controls.

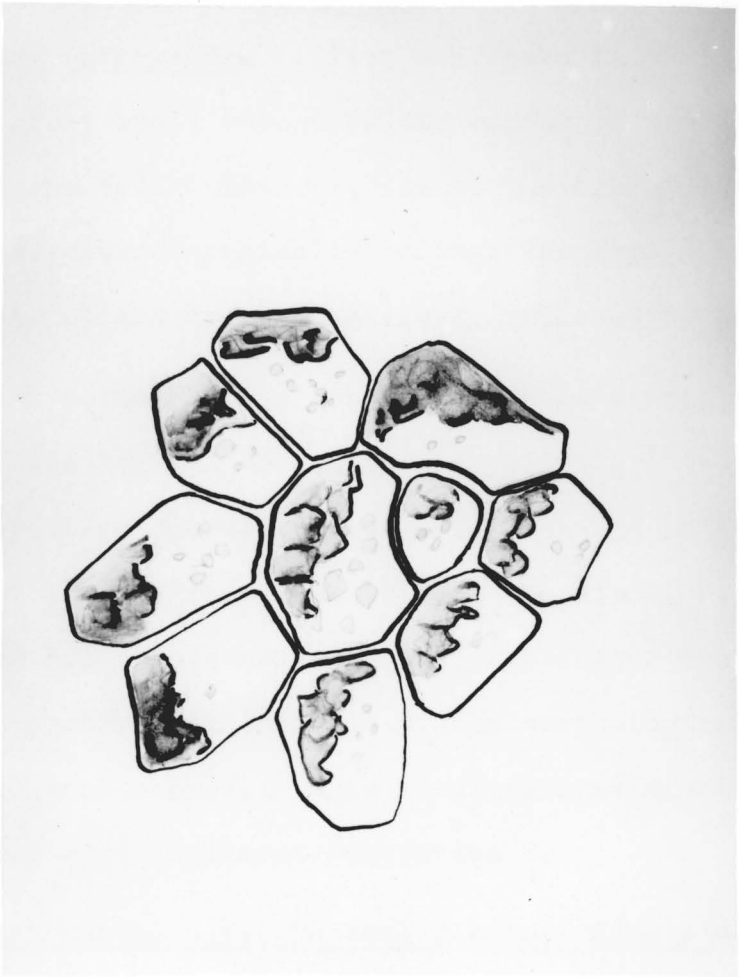


FIGURE 69 - Summer Generation 2. Cells of the Distal region after exposure to six weeks bright sunlight.

distal regions, accompanied by granule formation. There was a gradation of thallus colour in these plants similar to that illustrated in Figure 59.

Chloroplast morphology of Summer Generation 1 after six weeks treatment in the laboratory. After six weeks in the laboratory there was a comparatively small change in the colour of the plants of this generation (Figure 66). However, the morphology of the distal chloroplasts had altered radically in that the remaining area of homogeneous chloroplast had been entirely replaced by granules (Figure 67).

Had a great colour change accompanied this morphological modification, it would have been logical to conclude as Bliding (1944) had done, that chloroplast shrinkage caused thallus colour change in Enteromorpha. However, the similarity in colour between the distal regions of the treated and control plants suggests that the latter owed their colour to a change which occurred independent of the morphological alteration i.e. a physiological change. This conclusion is supported by the change which occurred in Summer Generation 2.

Chloroplast Morphology. Control plants; Summer Generation 2. In marked contrast to the control plants of Summer Generation 1, those of Summer Generation 2 were only about 3" long and rarely exposed directly to the sun. There was barely any detectable change in colour between the tip and base of the thallus. The homogeneous faintly granular chloroplasts of the basal and median regions (Figures 63 and 64 respectively) occupied between 70% and 100% of the lumen. In the distal region (Figure 65) however, some shrinkage and resultant granule production had occurred.

Chloroplast morphology of Summer Generation 2 after six weeks treatment in the laboratory. The colour of the treated plants (Figure 68) was comparable with similarly exposed plants of the first generation and thus represented a relatively greater colour change. It was surprising to find that this greater external colour change was accompanied by only a small alteration in chloroplast morphology (Figure 69). The change in this case appears to be entirely physiological.

Up to this point it has been established that thalli of comparable colour, belonging to the same population may be induced to change colour by exposure to strong sunlight. Before any conclusions regarding the reliability of this taxonomic character may be reached, it is necessary to demonstrate that thallus colour is entirely non heritable, which is the object of the following experiment. It was necessary to use plant material as uniform as possible in the following respects without growing it under controlled conditions.

(1) Plants at a very early stage in ontogenetic development and (2) of as deep shade of green as possible.

This uniform material was to be exposed to graded light intensities. If light was the colour-determining factor, a corresponding response in the form of a graded colour-change was to be expected.

Method. The winter generation at Motunau is established during the period, February - April each year. A number of identifiable stones* were therefore placed in the river at points A and E for the first three weeks of February 1966. This was sufficient time for plants of the

---

* The reason why more refined methods were not employed has already been explained.

winter generation at both placed to establish and grow to $\frac{1}{8}$" long. The colour of all these was a similar green. One stone from each point was then removed to the laboratory and placed in a 500 ml. beaker at the laboratory window.

The plants from point E were placed 1' away from the window where they received only one hour of direct illumination per day. Those from point A were positioned about 9" from the centre of the window where they received direct illumination for approximately 3 hours each day. At the time of writing (May 19, 1966) the plants near the centre of the window had grown another 2", those 1' away at the side $\frac{1}{8}$". In addition, the latter are all unquestionably much darker in colour than those plants nearer the light, with which they were originally identical.

There can be little doubt from these experiments that thallus colour is a purely environmentally controlled character.

Conclusions. In the section dealing with chloroplast morphology, two possible causes for colour changes in localised regions were suggested. (1) the fragmentation of the chloroplast or (2) a localised physiological change. Bliding (1944) found that shrinkage of the chloroplast altered the thallus colour. The following conclusions regarding the causes of chloroplast colour changes are based upon the experimental results cited in this and the preceeding section.

(1) Thallus colour is largely environmentally controlled. Under increasing light intensities the chloroplasts of young plants may be induced to occupy smaller areas of the lumen

(2) In the natural environment, moderate to deep green coloured plants may undergo a basipetal lightening of colour. The tips of these are normally floating at or near the surface of the water, and are therefore exposed to the most intense sunlight.

(3) The lightening of colour is caused by a physiological change within the chloroplast.

(4) In addition, exposure appears to hasten the normal process of plastid differentiation - shrinkage and granule formation (Bliding 1933, 1944; Gayral 1960).

(5) The writer cannot agree with Bliding's (1944) conclusion, that plastid shrinkage causes the colour change. It simply happens to coincide with the effective physiological change.

(6) On the basis of this evidence, the writer cannot agree with Agardh (1883), Setchell and Gardner (1920), or Chapman (1956) in their use of thallus colour as a taxonomic criterion. It is completely unreliable.

The Range of Variation in the Number of Pyrenoids and Width of the Mucilage Envelope.

Literature: Pyrenoids. 'The number of pyrenoids in the chloroplast is of great importance as a distinguishing character, especially in Enteromorpha, Ulvaria and Ulva (Bliding, 1933; Waern, 1952; Dangeard and other algologists of the Bot. Inst. of Bordeau)', Bliding (1963). E.g. Enteromorpha intestinalis rarely has more than one pyrenoid per cell while Enteromorpha clathrata 2 or more. In fact there is a constant characteristic number in several regions of E. clathrata, large old cells have 2 - several small pyrenoids, those at the base of the thallus 4, in the middle 2 - 3, and branchlets 2 (Bliding, 1944).

It is suggested in the literature that the number of pyrenoids increases in older regions of the thallus, as a result of plastid breakdown. Gayral (1960) working on Ulva linearis and Enteromorpha flabellata recorded that in older thalli the plastid became disorganised and starch grains accumulated in the cells. Bliding (1933) noted that in old zoospore producing plants of Enteromorpha clathrata the cells looked quite empty and the pyrenoids were not noticeable. However, he was able to identify both pyrenoids and starch grains in some E. clathrata zoospores (1933).

Hence there does not appear to be a general conversion of pyrenoids into starch grains in old plastids.

Results: Pyrenoids: Bluff population. From Tables 3, 4, and 5 it is clear that pyrenoids were visible in comparatively few cells of the 150 regions investigated in this population. They may not have shown up because the use of a starch-indicator stain was unsuccessful,

as explained earlier in this chapter. In addition, the structures labelled (p) in Figure 60, which were almost certainly pyrenoids, had a completely negative response to even strong iodine solutions. They were identified as pyrenoids only by their similarity to structures positively identified in other populations. It can only be concluded that in their present physiological state they completely lacked a starch sheath.

Results: Mucilage Envelope: Bluff population. The mucilage envelope was measureable in only a few plants. It appeared in these to decrease in thickness from the base to the tip of the thallus. If the cells remained in the same position with regard to the outside of the envelope in all individuals, it would appear that the thickness of the envelope is very variable.

Summary of chapters dealing with chloroplast morphology and orientation, chloroplast colour, presence (and number) or absence of pyrenoids, cell size, width of mucilage envelope, degree of convolution and its position on the thallus in a statistically significant sample of an Enteromorpha population at any one time of the season. All these characters with the exception of cell diameter may be expected to vary within wide limits. The diameter of cells in the Bluff population indicated that it belonged to one of 14 taxa of a total 47 recognised by Chapman (1956). In most plant groups a combination of moderately variable characters may be used to place any population into successively smaller units of any hierarchy. In the present population this could not be achieved because of the great overlap between the characters. Various reasons for this variability have been advanced in the sections dealing with individual characters.

However, it should be made clear at this point, that the problem is not insoluble, and that the solution is not very different from that for any other group of plants. The main requirement is one character to act as the 'main divisor' of the group. It is probable that cell diameter could be shown to serve this purpose if other populations were examined in a similar manner to that described here. Further, it is likely that the following could be used as differentiae, with certain reservations, in some groups: (A) Chloroplast morphology - if attention was given to the possibility of an ontogenetic cycle similar to that described herein. (B) Gross morphology - if attention was given to the complete range of variation of every population.

In contrast, the remaining two characters investigated do not appear to be of taxonomic importance: thallus colour - and here the writer is in complete agreement with Bliding (1963) and Womersley (1956) and the number of pyrenoids per cell. On this aspect the writer is in disagreement with Bliding (1933 - 1963), Waern (1952), Dangeard and the other Botanists of the Bot. Inst. of Bordeaux.

These views are based upon (and possibly limited by) evidence accumulated (only) from the study of South Island populations.

In many plant groups this would be adequate evidence upon which to base conclusions, but the inherent variability of the group necessitates caution. The possibility that the writer is yielding to the temptation of over-generalising his conclusions, should not be ignored. The South Island Enteromorpha populations may be the only ones in which many of the features described here occur. If such an assumption proved correct, then this alone could have caused the taxonomic difficulties encountered by the writer.

Strangely, this taxonomic uncertainty applied to all populations examined except that collected from the Motunau River, North Canterbury, in which a certain constancy of character was found. Some of these are compared with the type description of Enteromorpha intestinalis (L) Grev. in Table 12.

The Motunau population was classified as Enteromorpha intestinalis (L) Grev. regardless of the considerable discrepancy in habit. The reasons for this are discussed in a subsequent chapter.

All other populations were simply referred to by the name of the locality in which they were collected, e.g. 'the Bluff Enteromorpha

population'. Whilst this is clumsy, it is preferable to misidentifying species and interpreting the results of the following sections in an incorrect context.

In concluding this section, it is appropriate to draw several ideas together which have been put forward already, but under separate headings. This can be done most profitably in the form of a final justification for the writer's recognition of an ontogenetic sequence of several previously unrelated types of chloroplast morphology. It has not been possible to do this earlier without introducing ideas from several later sections.

Chapman and Bliding have described only single types of plastid for each species. If this were valid, their plants should be characterised by these during all stages of ontogeny. However, Gayral (1960) noted the occurrence in some species of starch accumulation and plastid fragmentation, associated particularly with old thalli. The present writer has expressed the view that:

(1) ontogenetic fragmentation of a homogeneous into a granular plastid similar to that recognised by Chapman (1956, 1961) is a normal part of the growth and development of the plant,

as are

(2) plastid shrinkage and localised colour changes which produce stellate and other forms of plastid.

Therefore the writer has included changes which occur during the entire life of the plant in constructing an ontogenetic sequence for the chloroplasts of various populations. However, in view of the other writers' interpretation it is also possible to consider some or even

all of these changes as the products of ageing.

Justification of the present writer's view depends on the following:

(1) These changes are found in plants of all ages.

(2) There are several types of granulation, but only a student very familiar with the group would realise that Gayral (1960) and Bliding are probably not referring to that described by this writer, which involves the cleavage of the plastid into a number of large rounded dark green bodies (each probably centred about a pyrenoid).

(3) Under intense illumination the colour of these dark green granules or a homogeneous dark green plastid may completely disappear, leaving a number of small pale blue iodine positive granules. It is probable that this is the change to which Gayral and Bliding are referring.

(4) However the main justification depends on determining whether or not the cells are physiologically active and therefore in fact not old. In discussing this further, it is profitable to draw an analogy between algae and higher plants.

A distinction may be drawn between senility, in which the cells have reached the state of vital exhaustion or degeneration, and differentiation. Recently chemical studies in higher plants have demonstrated that differentiated cells of several different types, are totipotent. Under the appropriate conditions they may produce a normal whole plant, regardless of their differences in morphology. Senile cells cannot behave in this manner.

TABLE 12 - A comparison of the Characters of E. intestinalis L. Grev. (Chapman, 1956) with those of a population from the Motunau River, North Canterbury.

	E. intestinalis	Motunau population
(1)	Thallus up to 1 m. long and 1 cm. broad.	Thallus up to 1.83 m. long and 5 cm. broad.
(2)	Frond simple, very occasionally branched.	Summer plants occasionally branched. Winter frequently.
(3)	Cells irregularly arranged.	Cells irregularly arranged.
(4)	Chloroplast filling the cell.	Chloroplast frequently filling the cell.
(5)	Full herbaceous green to semi transparent yellow green.	Dark green to pale yellow green.

The justification for recognising an ontogenetic sequence in algae rests upon the fact that the cells are not old, or senile, but differentiated. Every cell of an Enteromorpha thallus is capable of regenerating a new plant by some means. The holdfast cells are capable of forming new blades during early ontogeny (Dangeard, 1960) or considerably later (Delf, 1912). The cells of the blade may produce new plants by means of zoospores, gametes, or 'in situ' germination (Burrows, 1958; Lövlie, 1964; Dangeard, 1957, P.244). Thus cells containing a wide range of chloroplast morphology were totipotent, not senile.

In addition to these facts, the internal changes of the cell follow a cycle which is closely coupled to reproduction, not senility. In, e.g. Enteromorpha clathrata the cells at the tip of the thallus, which are amongst the first to become reproductive, have the greatest amount of starch (Bliding, 1944). During the 1 - 2 celled stages of ontogeny, pyrenoids are completely lacking in some sporelings (pers. obs.).

Regardless of the morphology of the chloroplast, it is always a part of any motile reproductive body. In view of the energy requirement during the motile period and early ontogeny (as indicated by starch losses), it is logical to assume that it is also functional. These features can only be considered as attributes of differentiated cells.

The writer would therefore advocate the use of this term in connection with any cell possessing what is here called a mature chloroplast. This would avoid any implication that the cell or a part of it is dead or dying, when this is not intended. As already pointed out, the change from an undifferentiated juvenile (homogeneous) chromatophore, to mature differentiated form, is so distinct that it may be termed heteroblastic.

CHAPTER 2 - LIFE HISTORY

THE SELECTION OF A SUITABLE CULTURE MEDIUM FOR USE IN THE SUBSEQUENT EXPERIMENTS

Introduction.

It has been said on various occasions that the number of algal culture media is almost as numerous as the number of algologists. A great number of the media may be used for culturing a wide range of algal species. For this reason, Droop (1960) suggested that the algae had remarkably stereotyped growth requirements. However, it is probable that one of the reasons for the great number of culture solutions is the limited knowledge of the physiological significance of the many naturally occurring nutrients.

Literature.

Comparatively little appears to be known of the precise role of the inorganic elements. Ca is definitely unnecessary, K is necessary and irreplaceable in case of the Chlorophyta. Both NH_4 and NO_3 may act as a source of Nitrogen, and occasionally NO_2, which is normally toxic when present in any but trace quantities.

With the improvement of analytical techniques shortly after the turn of this Century, a clear picture of the quantities of the various inorganic elements in sea water became available. As a result, numerous artificial sea water solutions were prepared, but no algae could be induced to grow in them. Allen (1914) was unable to grow diatoms without an addition of natural sea water or water extract of Ulva to his artificial solution. Similar results were obtained by Peach and

Drummond (1924) and Hervey (1933). It was apparent that something was missing from these solutions.

Accordingly Kylin (1943, 1946) investigated the influence of several inorganic micronutrient elements (all of which had been excluded by the previous workers) on the growth of algae in artificial media. In addition to the macronutrient elements (Na, K, Mg, Ca, Cl, SO_4, NO_3, PO_4) it was found that Bo, Zn, Cu, Mn, and Fe, were necessary in small quantities for growth to occur. Subsequently this list has been extended to include Mo, Co and Va (Meyers, 1962). Even with these additional elements, growth was considerably slower than in natural sea water, which Kylin (1946) believed to contain naturally produced growth promoting substances, in addition to the inorganic elements.

Concentrations of these substances were shown to be present in surface water and absent from that at a depth of 30 meters (de Valera, 1940), while higher concentrations still were found in the Fucus - Ascophyllum zone (Kylin, 1941; Levring, 1945). The influence of organic compounds in algal cultures was already known from the pioneer studies of Bouilhac (1897, 1898) and Treboux (1905). However, the great importance of such substances in nature was now realised.

By testing the effect of various extracts on algae growing in culture, growth stimulating substances have been demonstrated in soil extracts (Pringsheim, 1912, P.326), peat extracts (Wettstein, 1921) as well as algal extracts (Schreiber, 1935; Suneson, 1942, 1943). They are believed to be soluble iron compounds (Pringsheim, 1949) which Kylin (1946) suggested were transferred from the soil to the sea by rivers. Other organic substances also have a stimulating effect, e.g.

ascorbic acid, aneurine and heteroauxin (Kylin, 1942), and amino-acids (Baudrimont, 1960).

Natural sea water is therefore a balanced system of inorganic macro and micro nutrient elements, and probably a variety of growth promoting substances. The majority of artificial media in use today have a similar tripartite pattern of composition.

The Problems in Formulation of Artificial Media.

For the purposes of the present discussion, two types of artificial media may be distinguished:-

(1) completely artificial,

and (2) partly artificial media.

The use of completely artificial media is beset with more problems than use of the partly artificial media, neither being completely satisfactory. The solutions of Chu (1942) and Knop (Pringsheim, 1949) may be taken as examples of completely artificial media. (Chu's 1942 No. 10 medium is shown below).

$Ca(NO_3)_2$	.004%
K_2HPO_4	.001% or .0005%
$MgSO_4 7H_2O$	.0025%
Na_2CO_3	.002%
Na_2SiO_3	.0025%
$FeCl_3$	.00008%

The problems associated with this type of medium stem from:-

(1) techniques of sterilisation

(2) pH buffering

(3) chelating certain essential micronutrient elements.

The pH of natural sea water (at the surface), normally varies little from 8.1 - 8.2. This is maintained by buffers in artificial media which must be able to withstand autoclaving if sterile cultures are desired. T.R.I.S. is a relatively nontoxic buffer which maintains pH8. Organic acids with higher dissociation constants may be used when a higher pH is required, e.g. Valine pK_2 9.6, glycine pK_2 9.7. There are several objections to the use of buffers.

(1) They needlessly complicate formulae (Droop, 1960).

(2) Many may also act as chelating agents.

(3) Although they are introduced for physical reasons, many are metabolically active, and may therefore be utilized by the algae in various ways (Droop, 1960).

One of the most difficult elements with which to supply algae in culture is iron. In fact it is not understood how this element is obtained by algae at all in the natural environment. Under the alkaline conditions of sea water iron exists as ferric hydroxide, which is only available to plants in the smallest trace quantities. In artificial media, the supply of iron may be maintained by using a chelating agent, which forms a reversible complex with the metal cation. As the level of free metal decreases through absorption by the plant, the complexed iron is gradually taken into solution. Citrate and E.D.T.A. are both effective chelating agents, although x10 - x100 the amount of citric acid over iron is necessary. E.D.T.A. has the disadvantage that Ca and Mg have a strong affinity for it. However, it is not known how the supply of many of the other elements is influenced by the presence of any of the chelating agents.

Schreiber's medium may be taken as an example of the partly artificial type of culture solutions. The formula of 'Erdschreiber' Medium (the most widely used of any culture media) is shown below.

$NaNO_3$	1·0 mg
$Na_2HPO_4 10H_2O$	.2 mg
Sea Water	100 cc
Soil Extract	5 cc

This formula is considered partly artificial on the basis of the added excess $NaNO_3$, PO_4 as $NaNO_3$ and Na_2HPO_4. Soil extract probably provides greater quantities of organic substances than are present in natural sea water, which could also be considered an artificial aspect of the formula.

There are several problems associated with the use of this medium, caused mainly by:-

(1) sterilization

and (2) the maintenance of pH.

If sterile cultures are required, the sea water must be either passed through a bacterial filter or autoclaved, usually at 15 lbs per sq. in. for 20 minutes. According to Droop (1960) this caused the solubility product of the natural pH buffer ($CaCO_3$) to be exceeded, resulting in its precipitation, and an acid pH swing. However, the writer found autoclaving caused a negligible pH swing, but should a significant pH change have occurred, the use of a buffer with its attendant problems would have been necessary. The writer also found that the concentrations of 1·0 mg $NaNo_3$ and .2 mg Na_2HPO_4 indicated in the original (Schreiber) formula were considerably in excess of the

solubility of these salts at pH 8.1 in natural sea water. They were therefore reduced by half to avoid excessive precipitation.

Natural sea water is a delicately balanced system of inorganic elements and organic growth stimulating substances. Compared to natural growth rates in this medium, a moderate rate may be produced in artificial media in the presence of a variety of organic substances. Many of these are contained in soil and peat extract, in addition to such substances as pH buffers, chelating agents, and probably a variety of micronutrient elements. However, as the best growth occurs in the presence of other micro organisms it is not clear whether the stimulation is a direct or indirect result of the presence of organic additives.

Some experimental evidence is suggestive of an indirect relationship. In spite of the elaborate sterilisation techniques employed in many studies of algae, many members of the Ulvaceae appear to grow best in non sterile culture. Provasoli (1958) and Kylin (1942) found that Enteromorpha and Ulva would not complete growth of a single generation in sterile culture, even when supplied with various quantities of pure growth hormones. Føyn (1934) found that Ulva lactuca and Cladophora sp. grew successfully in non sterile culture solutions containing quantities of soil extract. By comparison with results such as those of Føyn, Provasoli (1958) concluded that the beneficial effect of soil extract was elicited by favouring the growth of bacteria and other contaminant micro organisms which actually produced the growth hormone(s).

Bold (1942) stated that the 'use of organic ingredients (soil and peat extracts, carbohydrates, acetates, etc.) in culture media is

unsatisfactory, and impossible in the presence of bacteria --- no conclusions of the relation of algae to organic matter are valid without bacteria-free culture work!" However, the present writer would disagree with this. The use of organic ingredients in the presence of bacteria is necessary for the Ulvaceae to produce a rate of growth practical for experimental purposes.

Culture Media used in the Present Experiments.

From the outset of these experiments, sterile cultures were not employed. The main reason for this was the fact that zooids could not be obtained in sufficient numbers. Pringsheim's technique (1921, P.402) for establishing sterile cultures requires the zooids to be placed in a considerable volume of water and repeatedly pipetted into quantities of sterile water. At no time did the writer obtain sufficient zooids to create such a favourable ratio of zooids to bacteria. Instead unialgal cultures, in which Enteromorpha was present in such amounts that its swarmers could not be confused with any other organisms*, were employed. Various types of culture media were used. The majority of experiments required only a practical growth rate - one not necessarily the same as that in the natural environment. An Erdschreiber solution, modified as follows, was found most satisfactory.

(1) The quantities of $NaNa_3$ and Na_2HPO_4 were reduced by half.

(2) The 5% soil extract of Schreiber was replaced in a number of cultures with a peat extract, which effectively reduced the growth rate of contaminant diatons by maintaining a slightly

---

* Terminology after Pringsheim (1921) modified by Smith and later Bold (1942

acid pH. When supplies of this could no longer be obtained, soil extract was used. The corresponding increase in the growth rate of contaminant organisms did not render the cultures useless until after the desired observations had been made.

However, these Erdschreiber cultures would not permit even one generation to be cultured to the stage of reproduction. After a considerable period of experimentation the solution was found. Fertile plants, from which cultures were desired, were left in a 500 ml vessel of filtered sea water. When zooids were released to establish the first generation the parent plants were left to decay, thus simulating the beneficial effects of algal extracts (Schreiber, 1935; Suneson, 1942, 1943). The second generation was established in the same manner, leaving the parent plants to decay. It was found that the growth rate of the second generation was greater than the first, which was still faster than in any modified Erdschreiber medium.

Successive generations could be kept separate with the aid of a little prior knowledge. As the plants approached reproductive maturity the water level in the vessel was raised. Thus a succession of generations could be separated at ascending heights on the vessel wall.

It is evident from the literature survey, and the results of the present experiments, that artificial media of any type are at best clumsy attempts to duplicate the natural environment.

THE PROCESSES LEADING UP TO ZYGOTE AND ZOOSPORE FORMATION

Introduction.

The first requirement for the establishment of cultures was a supply of reproductive bodies. According to Smith (1947) *Ulva* liberates zoospores and gametes every two weeks. To effect gamete release, Bliding (1963) gives the following directions ... "It is advisable to collect a well developed sea water material in the afternoon, isolate the specimens and keep them in darkness or feeble light. Next morning, early, they can be transferred into dishes with sea water, and then the mature gametangia are emptied, mostly at once. Only in a few species e.g. *Enteromorpha clathrata* are the swarmers not liberated in the morning, but afternoon". The writer followed this procedure with complete lack of success.

Therefore attention was devoted to the environmental conditions surrounding zooid release in the natural environment and laboratory. In order to understand the complete process, however, some attention was also devoted to the following subjects.

(1) Life histories in the Genus *Enteromorpha*.

(2) Colour differences between fertile and vegetative regions of the thallus.

(3) The ratio of gametophyte to sporophyte plants.

(4) Environmental factors which bring about zooid release
   (a) in the natural habitat
   and (b) in the laboratory.

(5) The cleavage pattern of the sporangial protoplast.

(6) The method of zooid release under both of these conditions.

(7) The structure of Enteromorpha intestinalis zooids, and the variation in structure of reproductive bodies throughout the Genus.

(8) The length of the motile period and behaviour of the zooids before and after copulation.

(9) Vegetative Reproduction - in situ germination.

Both the literature survey and presentation of results follow this order.

Literature.

Life Histories in the Genus Enteromorpha. The periods during which a particular type of reproductive body may be liberated is determined by a number of factors, including the life history. Most species may be classified as monomorphic diplohaplonts (E. intestinalis; Kylin, 1930, Bliding, 1948: E. clathrata; Bliding, 1944: E. compressa; Hartmann, 1929, Bliding, 1933, 1948a.: E. jugoslavica; Bliding 1960: E. Kylinii; Bliding 1948a.: E. prolifera; Bliding 1933, 1939: E. compressa var. linqulata; Ramanathon 1939: E. ramulosa; Hartmann 1929), with a sequence of haplaid gamete producing and diploid zoospore producing generations in a complete life history. The monomorphic diplontic species (E. Linza; Bliding 1933, 1939, 1944, Yamada and Saito 1939: E. ahlerniana; Bliding 1933, 1944: E. intestinalis; Bliding 1948: E. aragoensis; Bliding 1960) reproduce only by quadri-flagellate zoospores, while others (E. biflagellata; Bliding 1944 and E. adriatica; Bliding 1960) reproduce by bi flagellate zoospores frequently called

neutrospores. The monomorphic diplohaplonts therefore have a succession of zoospore and gamete producing generations in a complete life history, the monomorphic diplonts only zoospore or neutrospore producing generations.

Colour differences between fertile and vegetative regions of thallus. In both Ulva and Enteromorpha there may be colour differences between fertile and vegetative regions of a thallus. In anisogamous species of Ulva, e.g. U. lobata and U. angusta, fertile regions of male gametophytes are yellow to yellow tan, female gametophytes deep green (Smith, 1947). Colour differences have also been reported for Enteromorpha. Kylin distinguished 3 kinds of plants by the colour of the fertile region - male, orange-yellow; female, green-yellow; and zoospore producing plants darker greenish-yellow. There are no colour differences between fertile gametophytes of isogamous species.

The ratio of Gametophyte to Sporophyte plants. The ratio of gametophyte to sporophyte plants in Ulva populations on the Monterey Peninsula was about equal (Smith, 1947). However Kiyake and Kuneida (1931) found only eight gametophytes amongst hundreds of fertile thalli, while Yamada and Saito (1938) found the opposite - 3 sporophytes amongst 72 gametophytes. Moewus (1938) could find only sporophytes of Ulva Lactuca.

There are several possible explanations for these seemingly divergent reports.

(1) There could be a regular succession of generations of the one species each producing only zoospores or gametes.

(2) It has been shown that zoospores and gametes of at least 6 Ulva species are liberated at 14 day intervals, and that the period of gamete liberation starts four days later than zoospore release. If only short term observations were made in the laboratory, possibly one generation alone would manifest itself.

(3) Bliding (1933) found a population of Enteromorpha compressa which included only a few male plants. However, the uncopulated female gametes consistently developed parthenogenetically.

(4) Arasake and Shihira (1959) found plants of Enteromorpha Linza which liberated zoospores and normal gametes. They concluded that the type of reproductive body produced was considerably influenced by the prevailing environmental conditions. It is possible that plants which produce both gametes and zoospores originate from the apomictic development of uncopulated gametes.

In e.g. Ulva lactuca where this is known to occur (Føyn, 1931) the diploid chromosome number may be reinstated in some cells only. These may produce normal diploid spores repeating the diploid generation (recorded in E. intestinalis and E. compressa) or haploid spores developing into normal gametophytes (E. Linza). In cases where the parent thallus already contains haploid and diploid tissue, environmental influence need not be a prerequisite for the simultaneous production of both types of reproductive body.

In summary, gametes and zoospores may occur concurrently in the same environment, due to one of the following:-

(1) The existence of separate gamete and zoospore producing plants in the environment at the same time,

(2) Environmental influence on a thallus capable of producing zoospores or gametes,

or (3) the presence of apomictically produced thalli with both haploid and diploid cells.

Environmental factors which bring about zooid release in the natural environment. The factors which bring about the release of zooids in the natural environment are not at all well understood. There is no explanation why e.g. Ulva, Monostroma, and Dictyota (Smith, 1964) have a distinct 14 day periodicity and E. intestinalis a monthly one (Christie and Evans, 1962). While this behaviour is linked with the length of the tidal cycle, the manner in which this factor operates is obscure, because it has been demonstrated that Dictyota maintains its periodicity even when removed from the sea.

However, if the plants are maintained in culture for a number of generations, it is possible that the release mechanism may be impaired. Lerston and Voth (1960) could only effect zooid release in three Enteromorpha species from a culture collection by means of one of many experimental techniques they tested. By comparison, an unidentified species growing wild in a Chicago Marine Aquarium responded in various degrees to all stimuli.

Neither is there any explanation why zooids should be released at a specific time of the day. Schiller (1907), Føyn (1929) and Miyake and Kunieda (1931) all recorded liberation of swarmers from Ulva between 4.30 a.m. and 6 a.m. It is interesting to note that Yamada and Saito (1939) found that liberation occurred at any time during the day. Refer also to P.167 of this section for Bliding's observations.

The manipulation of the environment to effect zooid release in the laboratory. Numerous methods have been used to effect zooid release in the laboratory, (presumably regardless of periodicity). Some examples of these are given below:

(1) The fertile blades may be removed from water for an hour and reimersed (Smith, 1947).

(2) If no discharge occurs, the blades may be left in the laboratory to 'ripen' for a few days. A mere change of water is sufficient stimulus to effect discharge of plants treated in this way (Smith, 1947).

(3) Fertile plants may simply be kept in sterile water until zooid release is effected (Cauro, 1958; Gayral, 1960).

(4) Other workers have found that placing the fertile thalli in enriched sea water hastens zooid discharge (Lerston and Voth, 1960).

However, the change of external environmental conditions does not bring about zoospore formation with the regularity that has been claimed, and one cannot always be certain of obtaining zoospores at a desired time by modifying the environment (Klebs, 1896).

Description of Zooid Release in the Natural Environment. There appears to be only one detailed description of zooid release for the Genus Ulva - that of Smith (1947) "gametes and zoospores are discharged in a non motile condition, and as rapidly as they can stream one by one through the pore. They become motile 10 - 15 seconds after discharge, and within 30 seconds the mass disintegrates into individuals swimming in various directions ..... often one sees moving swarmers within many of the cells. This is thought to be abnormal and due to trapping of the swarmers by the cover glass pressing against the surface of the blade".

Cleavage of the sporangial and gametangial protoplast. The process of cell cleavage in Ulva (Smith, 1947) is the same for gametes and zoospores. The chromatophore shifts from the apex to the side of the cell immediately before division one. At the same time an apical papilla, through which the zooids will ultimately escape, appears on the outside wall. The second cleavage is parallel to the surface of the thallus, and the third at right angles to this. When gametes are formed, division into more and smaller units is synchronous within a single cell.

The structure of Enteromorpha intestinalis zooids and the variation in their structure throughout the Genus. The structure of only Enteromorpha intestinalis zooids need be considered in detail, since most of the subsequent discussion is restricted to this species. The average dimensions of the male gametes are 6.1 x 2.0μ, the female gametes 6.8 x 3.7μ, and of the zoospores 10.0 x 5μ.

The chloroplasts are better developed in the zoospores, while both types of zooid may possess pyrenoids, smaller starch inclusions and an

eye spot (Bliding, 1963). Within the limits of these size ranges a small amount of variation from the usual 'pear-shape' is possible.

Burrows (1958 a.) showed that storage of fertile thalli at 2^{0}c in dim light caused the production of large compound gametes with "multiple groups of flagellae". She postulated that abnormal swarmers may be a means of tiding over a period of unfavourable environmental conditions without sexual reproduction. The number of flagellae on a single zoospore of E. Linza was found to vary from 1-5. Arasake and Shihira (1959) concluded that this was to some extent environmentally controlled.

The length of the motile period and zooid behaviour. The motile period for zoospores in Ulva species may last up to 24 hours, but that of the gametes is never as long (Smith, 1947). The termination of this period in zoospores is preceeded by a change from positive to negative phototaxis. In Enteromorpha intestinalis this occurs 30 - 40 minutes after liberation (Kylin, 1930). The gametes of Ulva and Enteromorpha remain positively phototactic throughout the entire motile period.

Fusion is preceeded by gamete clumping in all species of Ulva investigated (Smith, 1947) and several Enteromorpha populations. Under conditions of increasing salinity, Moewus (1948) was able to induce fusion of more than two gametes, producing triploid and tetrapolid zygotes.

The ontogeny of the germling is reviewed in a subsequent chapter. The following points regarding the practical establishment of cultures may be concluded from this literature summary to this point.

(1) In practice there should be no great difficulty in identifying fertile plants,

(2) or effecting release of their zooids in the laboratory.

Vegetative reproduction - in situ germination. In a monomorphic diplohaplontic life history, the gamete producing haploid generation reproduces sexually, and the diploid zoospore producing generation reproduces by asexual means. However, there has been a gradual recognition of the fact that members of the Ulvaceae may reproduce by vegetative processes in addition to the production of zoospores.

Our first knowledge of this aspect of reproduction was apparently gained when Schiller (1907) and Delf (1912) found that individual rhizoids of an Ulva holdfast were able to give rise to whole new blades each spring. Delf (1912) suggested that the multinucleate character of these rhizoids (Thuret, 1878; Delf, 1912; Carter, 1926) was associated with the perennial habit of the holdfast. This theory still appears sound in light of more recent evidence. Carter (1926) demonstrated that the holdfasts of Monostroma, which are seasonal to the best of the writer's knowledge, were composed of uninucleate rhizoids. Later it was found that the uninucleate blade cells were totipotent without the intervention of zooid formation.

Under cultural conditions, enlarged vegetative cells of the stipe (Løvlie, 1964) and blade (Dangeard, 1957 b.; Provasoli, 1958a.) of Enteromorpha may give rise to complete new plants. The cleaved products of the zoosporangium may also germinate in the same way (Dangeard, 1955). The same author also found that the side of fertile

E. intestinalis blades in contact with estuarine soil were stimulated to germinate in this way rather than by the release of motile zoospores. Dangeard (1955) believed that this particular environment caused the loss of the normal reproductive powers and substitution of vegetative multiplication.

The present writer applies the term 'in situ germination' where:-

(1) a whole vegetative cell has given rise to a new plant while still attached to the parent,

and, (2) the cleaved products of a gametangium or zoosporangium develop into a new plant without being discharged.

The reason for the use of the term 'in situ germination' is the writer's discovery that gametes as well as zoospores could behave in this way. This does not appear to have been previously recorded. In the case of the gametangium, a distinction between "vegetative" and sexual reproduction could only be made if the products of cleavage* did not fuse within the cell. The writer is not certain that they don't. The use of the term 'in situ germination' is to be preferred to 'vegetative reproduction', as it only describes a process, and avoids any other implications.

Vegetative reproduction is commonly well developed in estuaries, and occasionally other habitats, throughout the world. The unique

---

* The gametes can hardly be termed this when they are never released from the gametangium in a motile condition to fuse with one another.

environmental conditions are not only responsible for distinctive forms of Chlorophyta e.g. Enteromorpha intestinalis, (Dangeard, 1955) E. clathrata ecad prostrata Le Jol, (Chapman, 1956) but also Phaeophyta e.g. Fucus Vesiculosus ecad volubilus and Asocophyllum ecad scorpioides and ecad mackaii, (Chapman, 1960) and Rhodophyta. Chapman (1960) suggested that in the Phaeophyta the high relative humidity prevented the cell sap from reaching a critical concentration and thus prevented formation of sexual organs. The writer feels that the reason for the prominence of in situ germination in estuarine E. intestinalis populations may be simpler than this. It is possible that with the short period of zooid motility and extensive areas of mud, there is a great chance that the reproductive bodies would become embedded in the mud, which is completely unsuitable for further development. However, if the plants grew to e.g. a size of 6 - 12 cells while still attached to the parent, it is likely that they would float upon release. In this way they could be transported until they touched some solid surface to which they could attach, and continue their development.

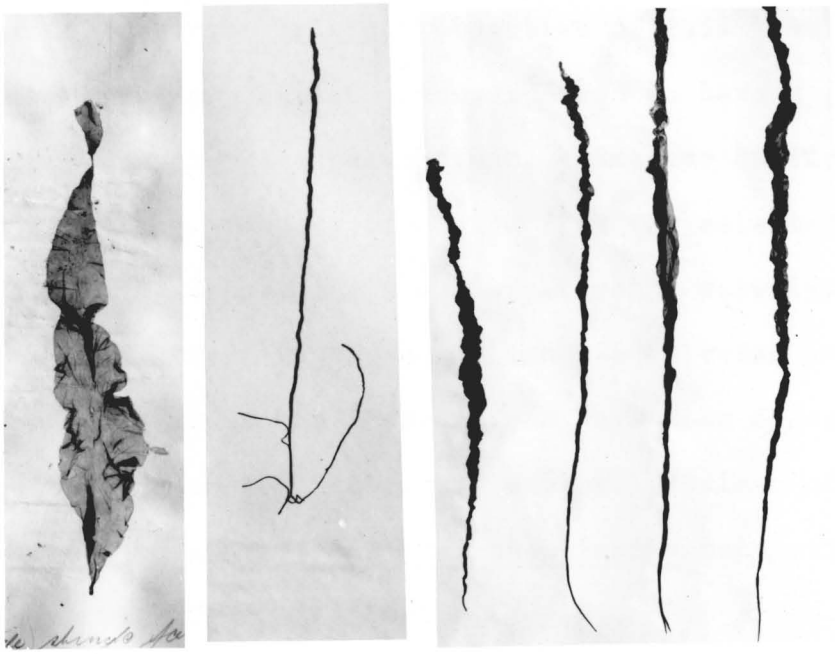


FIGURE 70 - Plants illustrating various points in the range of gross morphology of the summer generation.

FIGURE 71 - Plants illustrating the range of gross morphology of the winter generation at Motunau.

Results of the Present Study.

As already pointed out, the original objective of this thesis was to culture any Enteromorpha population which appeared to have a dimorphic life history, through two generations. Extreme difficulty was experienced in obtaining a supply of zooids from the selected population. The following observations and experiments were largely made while the writer was attempting to establish the cultures essential to this study. For convenience the observations have been organised into the same subject order as the literature survey. Unless otherwise stated, they all pertain to an Enteromorpha intestinalis population growing in the Motunau River in Canterbury.

The periodicity of Enteromorpha intestinalis in the Motunau River, North Canterbury. There appears to be a succession of distinct generations of the one species at this locality. In March 1965 - the summer generation (Figure 70) died and was completely removed by decay and tidal action several weeks before the winter generation (Figure 71) appeared. In September of that year, there was again an interval between the disappearance of the winter and reappearance of the summer generation. However, at the end of the following summer, in April 1966, the changeover was not quite so distinct. It began earlier, in February, when the summer generation disappeared quite suddenly from the seaward end of the river. However, it was not until June that all the summer plants had disappeared from the up-river end.

Nevertheless, there is an overall distinct seasonality or periodicity in this population, and a predictable time of changeover between each generation and the next. The writer is not aware of any

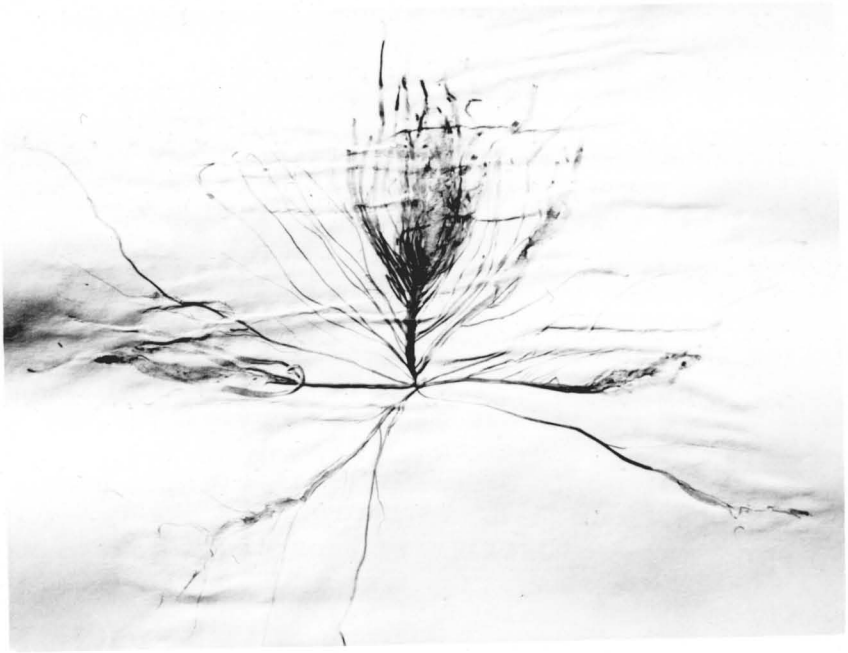


FIGURE 72 - A summer generation plant from Motunau which released gametes and zoospores during autumn.

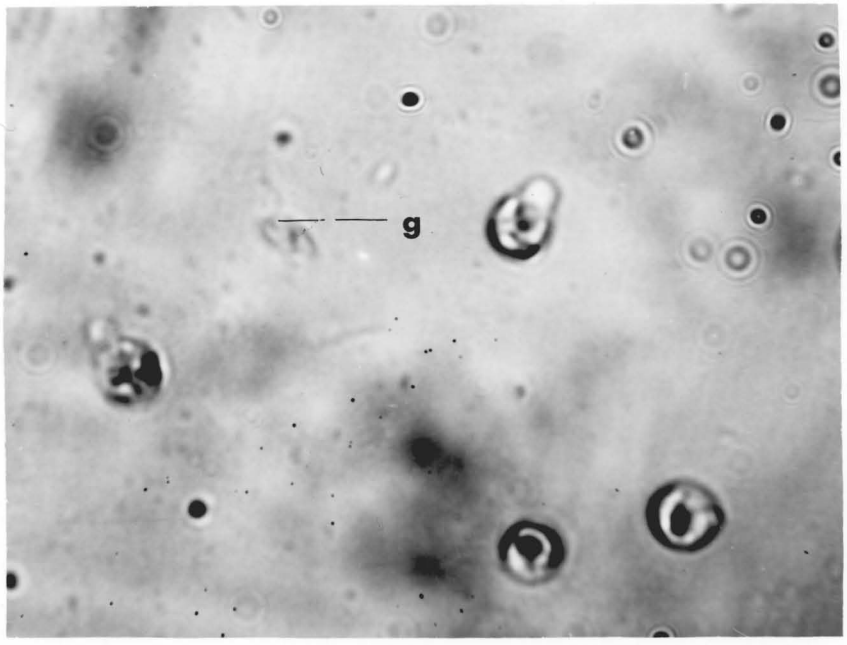


FIGURE 73 - Zoospores and a gamete (g) released from the summer generation plant in Figure 72. The poor quality of this illustration is due to the $\frac{1}{2}$ second exposure of the photomicrograph.

previous reports of such an occurrence for any other Enteromorpha population.

During the summer growing season new plants appear to be established only at certain times. The writer could not determine whether this was caused by (1) release of zooids at regular intervals or (2) the existence of particularly favourable environmental conditions at these times. The flush of new plants which appeared during the period December - January, was so distinct that it was termed Summer Generation 2. This distinguished the plants established approximately half way through the growing season from those established at the beginning which were still present. The writer recognises the possibility that this may simply reflect a situation peculiar to one summer. A comparable situation was not found in the winter generation.

Colour differences between fertile and vegetative regions of thallus. Where zoospores are formed, the thallus changes from pale green to a shade of olive green, which the writer would never consider as yellow green (Kylin, 1930). However, the mere fact that description of colour is very subject to human opinion makes it most unwise to attach great significance to this discrepancy.

There were no apparent colour differences between the anisogamous fertile and vegetative gametophyte thallus regions in this population.

The ratio of gametophyte to sporophyte plants. In many studies populations with an overwhelming dominance of gametophyte or sporophyte plants have been described for which there are three possible explanations.

(1) The gametophyte and sporophyte generations may occur independently at different times of the year. By collecting mature plants regularly from December 1964 to March 1966 it was found that most winter generation plants produced only gametes while those of the summer generation produced only zoospores. The exceptions to this are described below.

(2) That separate gametophyte and sporophyte plants grew together but released their gametes or zoospores at different times.

(3) Gametes and zoospores could be produced on the one thallus, but released at different times.

From the following observations, it is apparent that all of these explanations may apply to the Motunau population. Some plants of the summer zoospore-producing generation also produce a small number of gametes, and it is thought possible that some plants of the winter gamete-producing generation are also able to liberate zoospores.

The plant of the summer generation in Figure 72 was collected on 14.11.65 from point B. The following day it was placed in a beaker of sterile sea water. Two days later zoospore release occurred, and four days after this gametes were observed being released from sporangia in a region of thallus which had already liberated zoospores (Figure 73). On one occasion only was the release of gametes observed from regions which had not liberated their zoospores. Gametes produced in this manner in no way differed from those produced by the winter generation and could not copulate amongst themselves.

On 13 September 1965 fertile plants of the winter generation were collected from point A. These were cleaned, and left without water

in a sealed bottle for two days, to stimulate the effect of prolonged exposure in the natural environment on zooid release. Four days later they were returned to sterile sea water. Zoospore release occurred about 4 hours later, and extensive gamete release 2 days after this. It could not be determined whether both types of zooid were released from the same plant.

In summary it may be said that although some plants of the summer generation are able to produce gametes and zoospores, this generation is predominantly a zoospore producing one. The winter generation, on the other hand, is predominantly gamete producing, but either some whole plants, or certain regions of gamete producing plants are able to produce zoospores. The writer inclines to the view that there could be a small number of completely zoospore producing plants, established by copulation of the few summer gametes. This appears to be the first report of a distinct alternation of summer zoospore and winter gamete producing generations of Enteromorpha intestinalis.

The manipulation of the environment to effect zooid release in the laboratory. The literature survey has already touched upon the subject of zooid release in the laboratory, from which it could be concluded that there was no difficulty in this. Such was not the case. For almost a year the writer could not obtain the release of zooids in quantities large enough to permit even the beginning of cultural experiments.

The first difficulty encountered was that of identifying fertile thalli in the field. Continual visits to a population of Enteromorpha sp. growing in the Heathcote Estuary convinced the writer that a change of colour was not always associated with fertile regions of thallus.

These plants became reproductive without a colour change, and in addition, the release of zooids confirmed in the cells, though occurring in the natural environment, could not be effected in the laboratory. The unsuccessful attempts at this are detailed below.

Methods which were unsuccessful in effecting zooid release

(1) The fertile plants were transferred to the laboratory and viewed under the microscope. A gentle pressure upon the cover slip failed to effect zooid discharge.

(2) The blades were removed from water for an hour and reimmersed (Smith, 1947). Exposure to the air even for several hours similarly failed.

(3) Temperature shock treatments:

(a) Gentle heat applied to a blade collected as in (1) above.

(b) The fertile material was placed in a bottle of sea water and maintained at 9°c overnight. The following day it was warmed gradually by the heat from a 40 watt bulb.

(c) The fertile material was treated as in (b) above, with the exception that the plants were kept in a plastic bag without water.

(d) Plants transferred to the laboratory in sea water, briefly washed in sterile sea water at 18°c and immediately immersed in water at 22°c.

(e) Plants treated as in (d) above but transferred to water at 30°c. A few lethargic gametes were released on one occasion. Repetition of the treatment proved negative.

However, it was finally discovered that zooids could be released in the laboratory when the fertile plants were collected at a particular time of the season. Fertile plants collected in March, August, or September, December and February, released zooids:-

(1) immediately following collection,

(2) when treated in several ways,

and (3) over a wide range of laboratory environments.

Plants of the winter generation released zoospores and gametes when they were returned to water 5 days after collection. They also released gametes when the period out of water was omitted, although there was a 1 - 2 day lag period in this case, as the following experiment shows.

On September 14, 1965 a number of fertile plants were collected from point B in the Motunau River, and transferred in sea water to the laboratory. They were removed from this container on the following two successive days, freed of epiphytes with a camel hair brush and immersed in a beaker of autoclaved sea water at the laboratory window. Zoospore release occurred three days after the transfer in all cases. This suggests that zooid release may be related to an intrinsic periodic factor independent of any laboratory treatment.

During the above-mentioned periods of the year zooids could be obtained with regularity in the laboratory in two ways. Not only could zooids formed in the natural environment be released, but vegetative plants could be collected and zooid genesis and release be effected in them.

In February, 1966 six large plants, the purely vegetative state of which was confirmed by a microscopic examination, were collected from

	4 1.30 p.m.	5 1.00 p.m.	5 5.30 p.m.	6 1.30 p.m.	6 5.30 p.m.	9 5.30 p.m.
1	large number of zoospores released			some gametes released		
	80°F	70°F	82°F	71°F	85°F	91°F
	280 F.C.	250 F.C.	200 F.C.	200 F.C.	250 F.C.	200 F.C.
2	zoospores released	no zoospores or gametes released				
	86°F	70°F	82°F	76°F	85°F	93°F
	280 F.C.	250 F.C.	200 F.C.	200 F.C.	250 F.C.	200 F.C.
3	zoospores released		some gametes released	some gametes released		
	85°F	70°F	77°F	72°F	86°F	89°F
	280 F.C.	250 F.C.	200 F.C.	200 F.C.	250 F.C.	200 F.C.
4	zoospores released	small number of zoospores released	small number of zoospores released	abundant gamete release	abundant gamete release	gametes still being released
	74°F	68°F	78°F	69°F	86°F	85°F
	290 F.C.	240 F.C.	170 F.C.	200 F.C.	290 F.C.	200 F.C.
5	zoospores released		some zoospores released	large number of gametes and zoospores released	gametes only released	gametes only released
	68°F		72°F	69°F	71°F	81°F
	290 F.C.		75 F.C.	200 F.C.	100 F.C.	125 F.C.
6	zoospores released		a few zoospores released		few zoospores released	gametes only released
	68°F		72°F	66°F	78°F	83°F
	190 F.C.		75 F.C.	200 F.C.	125 F.C.	125 F.C.

TABLE 13

point B in the Motunau River. They were treated exactly as those above. Three days after collection extensive areas of the thalli were transformed into sporangia, and on the fourth day zoospore release occurred. In order to determine the range of environmental conditions under which sporangia would discharge, 6 plants were collected on 5.12.66 and placed in beakers of sea water arranged in various positions about the window. In this way a considerable variation in the duration and intensity of light falling on the cultures, and variation in water temperature was created between the various vessels.

Records were made of the light intensity, water temperature and number of zooids released 4, 5, 6, and 9 days after establishment of the cultures. The higher water temperatures correspond with those cultures which received longer periods of more intense illumination. The results for the 6 plants are shown in Table 13 opposite.

Gametes were formed and discharged when the temperature was anywhere between 69°F and 86°F, and the light intensity between 100 and 200 foot candles. Zoospore release occurred within the temperature range 68 - 85°F and light intensity range of 75 - 290 foot candles.

In the natural environment gametes are normally associated with the winter and zoospores with the summer. It is therefore appropriate at this point to consider the relationship of the zooids to the environment when both kinds are produced by one plant at the same time of the season. If this plant was diploid, (1) both zoospores and gametes could have been products of meiosis and possessed the haploid chromosome number or undergone mitosis and remained diploid. These zooids could develop in a variety of ways, establishing any one of the following winter generations.

(1) Haploid zoospores would develop normally into a gametophyte generation, while diploid zoospores would repeat the sporophyte generation.

(2) Diploid gametes developing by apomixis would also repeat the sporophyte generation, while their copulation would lead to the establishment of polyploid plants.

(3) Haploid gametes developing without fusing would give rise to the gametophyte generation as normal haploid zoospores would do.

(4) The copulation of haploid gametes would lead to the development of another sporophyte generation, omitting the normal gametophyte phase.

In the experiment just cited, there was no marked difference between the response of zoospores and gametes to environmental factor fluctuation. The writer therefore inclines to the view that both were probably functionally and genetically similar. Thus the normal haploid zoospores would develop into the winter gametophyte generation, while the gametes developed into the same generation without fusion (apomixis).*

---

* Attempts to investigate the cytogenetic aspects of this problem were completely unsuccessful. Both the published procedures and numerous variations of the following nuclear staining techniques were attempted. (a) Aceto-carmine and aceto-orcein stains (Gurr, 1957), (b) Heidenhain's iron-alum haematoxylin (Ramanathan, 1939), (c) Godward's (1956) technique.

Plants collected at other times of the year than those mentioned in the above paragraphs have remained healthy but vegetative in the laboratory for longer than 6 months. There can be no doubt that (1) the condition of the plants on collection, and (2) the time of the season when collection is made has a great bearing on the behaviour of the plants in the laboratory. The writer is convinced that (1) the various methods for effecting zooid release recorded in the literature work only when the plants are in the right condition, and (2) that during this time there are a great variety of stimuli which may be effective in bringing about release. (3) Therefore, any precision advocated in published release techniques appears entirely unnecessary.

When plants are grown to maturity in artificial culture, it is possible that some modification occurs to the control mechanism of zooid discharge. Lerston and Voth (1960) found that three species of Enteromorpha from a culture collection would not discharge zooids in response to a variety of environmental stimuli, to all of which a wild species was able to respond. Gayral (1960) found that when Ulva linearis and Enteromorpha flabellata were cultured, they liberated zooids in small numbers over a period of time. The writer found that the third generation of Enteromorpha intestinalis cultured in natural sea water behaved in the same way. The explanation for these results may be as follows.

Under the constant laboratory conditions the algae are removed from the periodic phenomena of the natural environment, e.g. tidal cycles. With each successive generation there may therefore be a decrease in capacity to respond to these stimuli. If these were responsible for

the initiation of zooid formation as well as their liberation, the condition could arise in which the algae reproduce entirely vegetatively. Lerston and Voth's species obtained from the culture collection might well have been approaching this condition. Gayral (1960) suggested that the constant laboratory conditions were responsible for the longlevity of plants maintained in artificial culture, but not the modification to their period of zooid release.

The pattern of cleavage of the sporangial and gametangial protoplast. In the literature mention is not made of the relationship of the cleavage* plane, cell shape and zoospore shape and size.

The pattern of cleavage in Enteromorpha is apparently not as regular as that in Ulva. Frequently more walls are formed in one plane than in the other (Figure 74). In other cases one half of the sporangium may cleave regularly, the other half irregularly (Figure 74). From these figures it is clear that the cleavage planes are not always formed at right angles to one another, and neither does the sporangium always produce an even number of zooids.

The variation in zooid size is a direct result of the variable orientation of the cleavage plane in cells which vary in size and shape. In the top cell of Figure 74, most of the cleavage planes traverse the widest portion of the cell, while in the lower cell most formed across the narrow portion. The zoospores are therefore large and small

---

* The writer uses the term cleavage exclusively for partition of the whole protoplast, and the term division for the dividing nucleus.

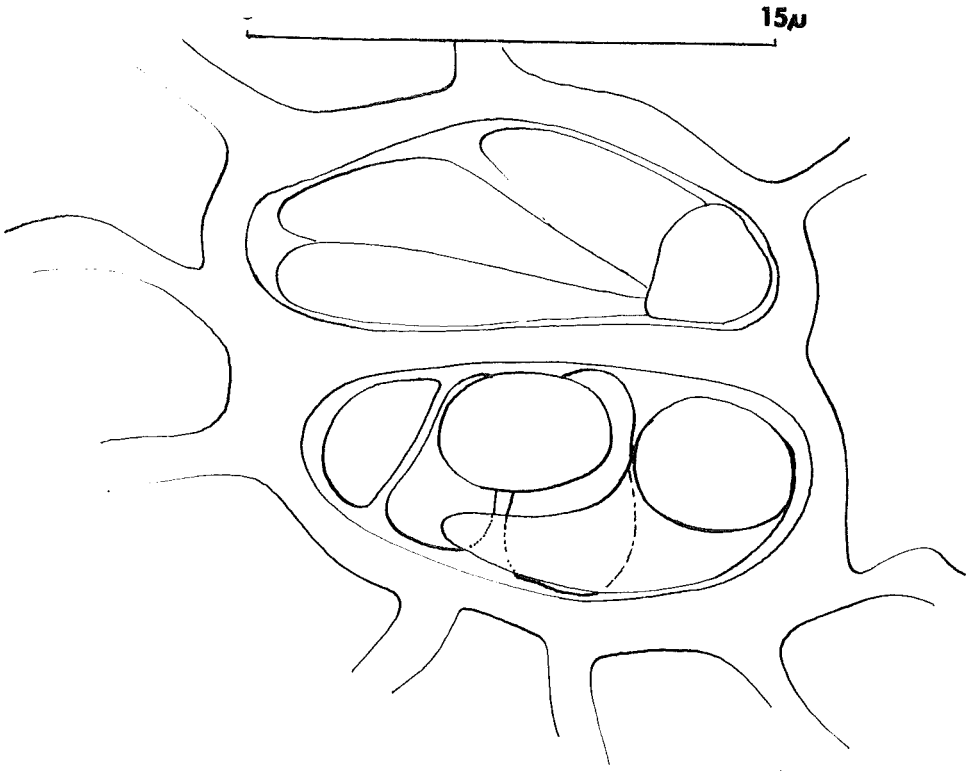
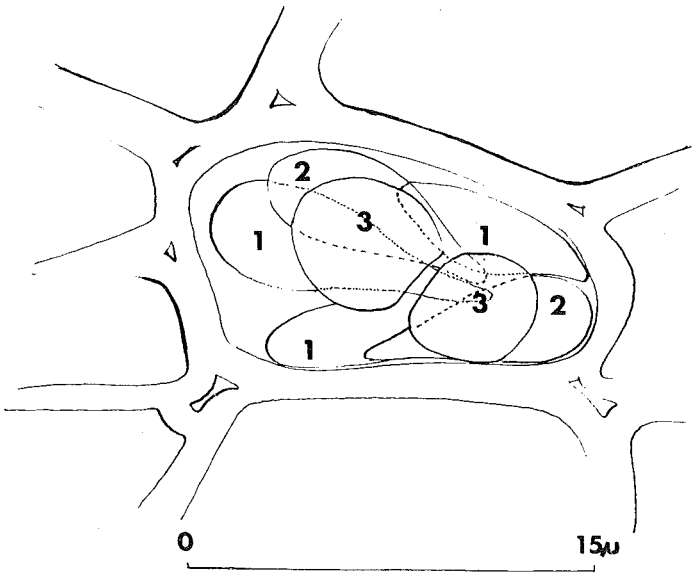


FIGURE 74 - Showing the variation in orientation of the cleavage planes in Enteromorpha intestinalis zoosporangia.

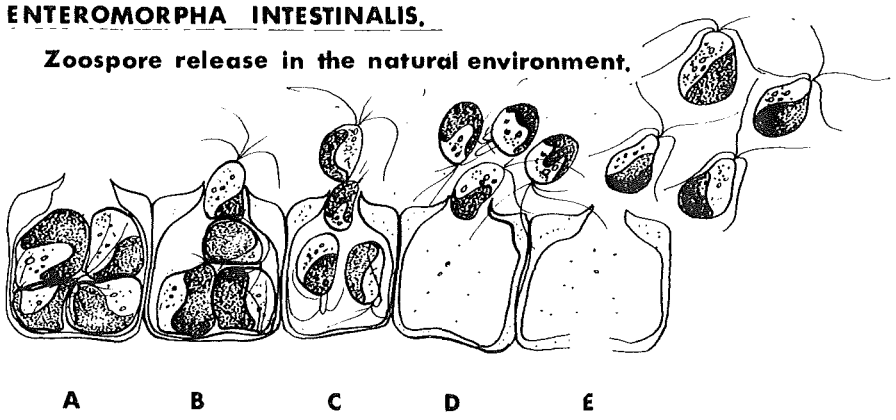


respectively. Although this appears as a statement of the obvious, rather surprisingly it has never been described as the explanation for size differences between zooids which have been taken for granted ever since this group has been studied.

The manner in which zoospores are released in Enteromorpha intestinalis (L.) Grev. There appear to be at least two possible methods of zoospore release in this species, one of which has only been found in plants raised in culture, the other occurs in plants collected from the natural environment. The latter, natural method of release is described first.

On June 6, 1966 a number of fertile plants of the summer generation of Enteromorpha intestinalis were collected from point B. The following day these liberated 'zoospores' very quickly, compared to the cultural method, and in a manner similar to that described for six Ulva species by Smith (1947), (P. 173 of this thesis). However, after half an hour they were still non motile, and the subsequent application of a heavy concentration of iodine revealed that they were completely aflagellate. However, on many other occasions the rapid liberation of motile quadriflagellate zoospores in a similar manner has been observed (Figure 75). The writer has never observed the liberation of non motile flagellate zooids.

Under cultural conditions, the formation of a papillate outgrowth on the outside wall of the cell has been observed. The zoospores become active inside the sporangium prior to release. The first zooid to leave enters the papilla posterior end first, and creates an exit canal through the cell wall and mucilage envelope for the remaining

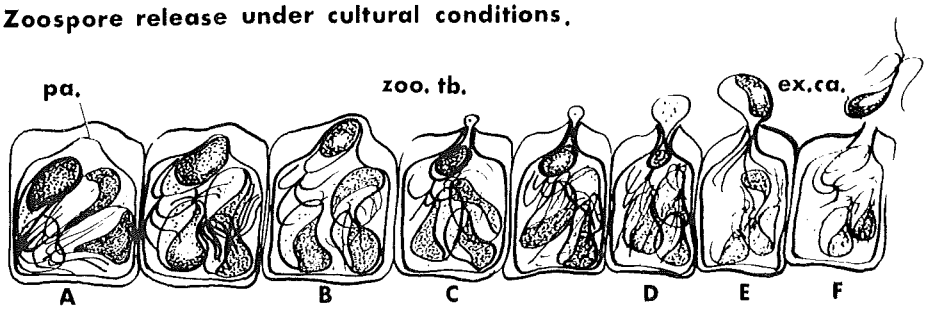


(A) A hole is formed (by an unknown means) in the outside wall which the zoospores pass through singly (B-D). Upon release they immediately swim away (E).

FIGURE 75

ENTEROMORPHA INTESTINALIS.

Zoospore release under cultural conditions.



(A) During sporogenesis, a papillate outgrowth forms on the outside wall of the sporangium. (B) The first zoospore released creates the exit canal (ex.ca.) for the rest. (C) It does this by forcing a tubular extension of its own plastic body - here termed a zoospore tube (zoo.tb.), through the wall of the sporangium. (D) Its cytoplasm is forced first through this canal, (E) followed by the plastid and flagellae. (F) The remaining zooids leave in the same way, but through a preformed exit canal.

Release of abnormal zoospores.

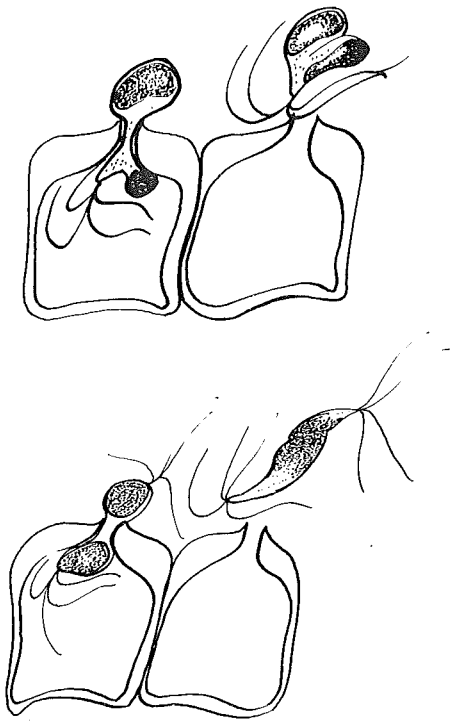


FIGURE 76

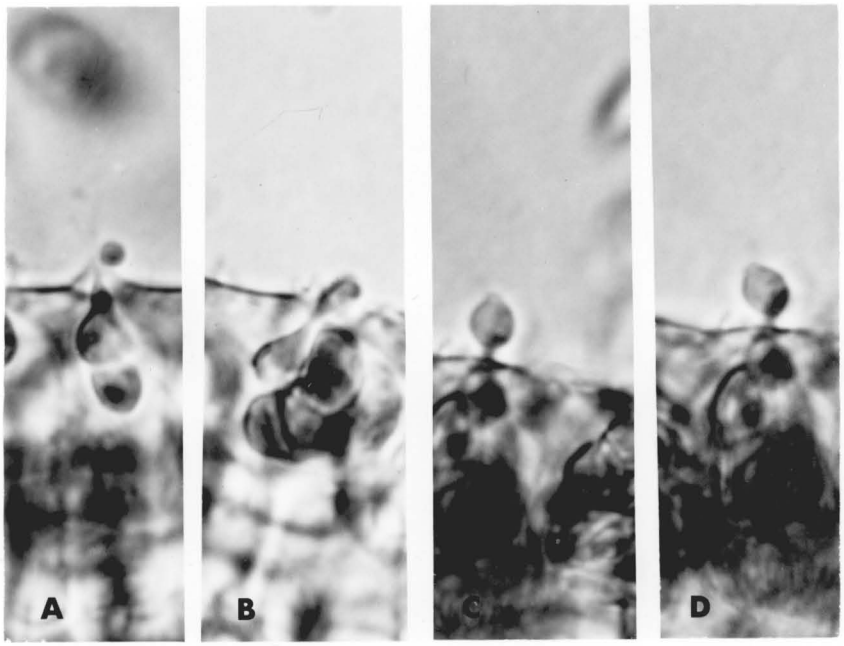
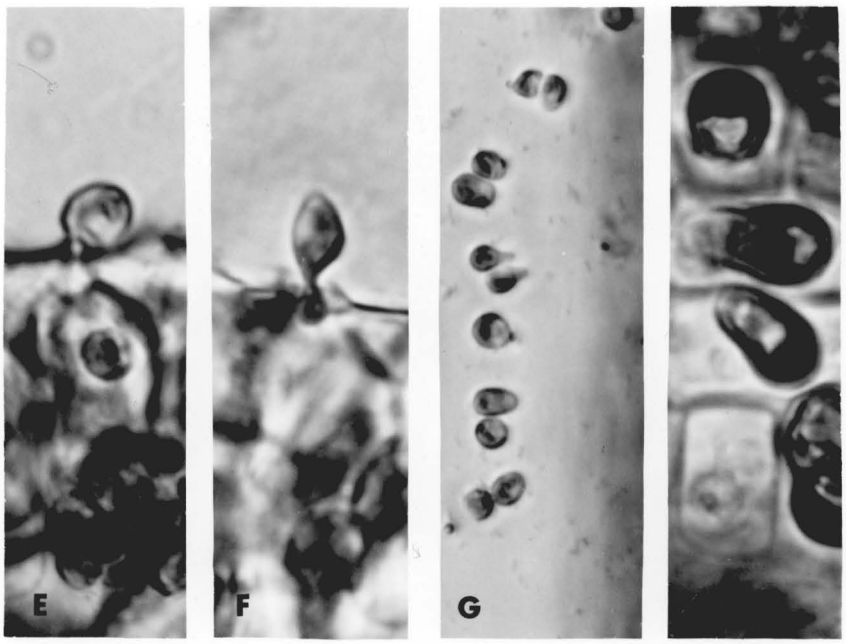


FIGURE 76 A - H - Zoospore release in culture. This photographic record is not a sequence of the release of a single zoospore. (A) formation of the exit canal, (B - F) through which the zoospore passes. (G) Some zoospores remain attached for a time to the inside of the sporangium by their flagellae, (H) while others are not released and germinate in situ.



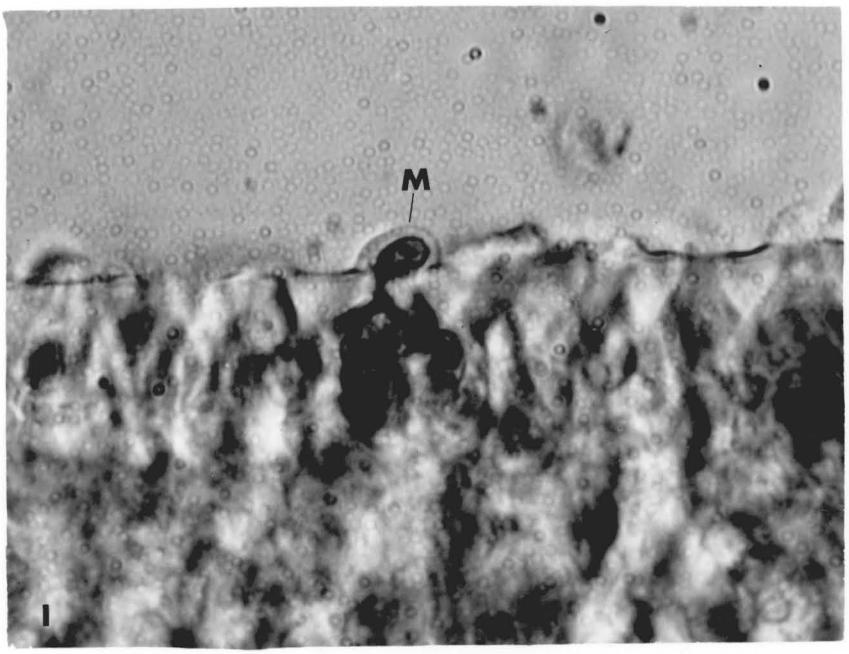
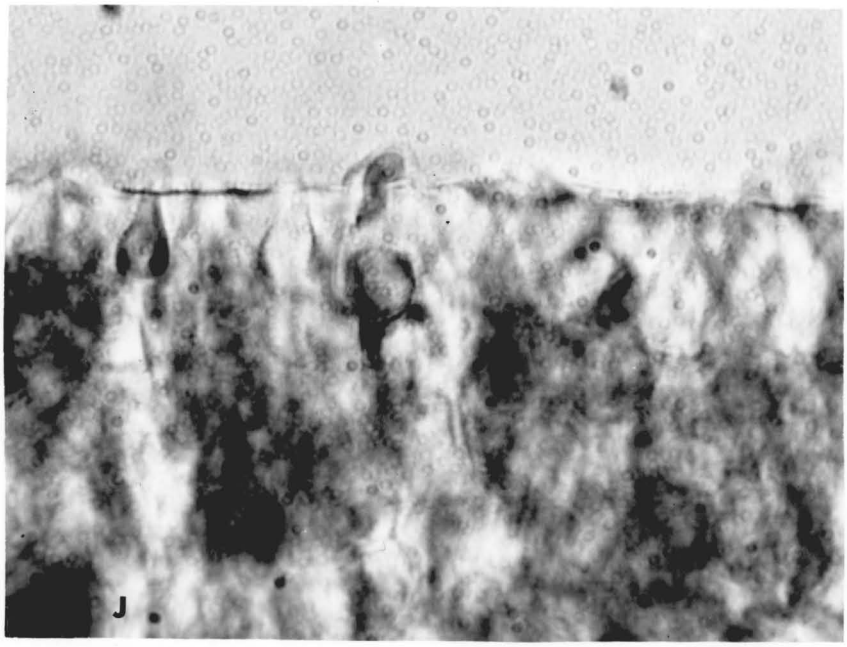


FIGURE 76 I - J - The plasticity of the mucilage envelope (m) is illustrated in (I) above. This could present a considerable obstacle to the zoospore, and explain why some fail to be released (J).



zoospores. This is accomplished in the following manner.

A tubular extension of the zooid wall termed a "zoospore tube" is formed between the papilla and outside of the sporangium. The cytoplasm of the zoospore is gradually forced into the tube causing the gradual expansion of the distal end. The chromatophore then passes rapidly through the tube, which promptly withdraws the flagellae from the sporangium. Their arrival at the outside causes the bubble of cytoplasm to assume the original shape of the zoospore instantly, which immediately turns about and swims off. All zoospores leave in the same manner, because the exit canal is always too narrow to allow their full width through (Figure 76 and 76 A - J).

The length of time a zoospore takes to leave the sporangium varies according to its size and shape. A small normal shaped zooid can pass through in approximately two seconds. Many of the larger abnormal ones take anything up to 60 seconds, but those with flagellae at both ends may take considerably longer, (Figure 76).

This method of release does not appear to have previously been recorded for any member of the Ulvaceae. Yamada and Saito (1938) published the following report of zoospore discharge from the zygote in Monostroma angicava Kjellm. "But sometimes the spores are discharged through a slender tube coming out from the surface of the zygotes, which can elongate to about 1 - 3 times the diameter of the zygotes".

The writer interprets this as meaning that the length of the tube is 1 - 3 times the diameter of the zygote. It is also possible that the spores elongated, although their length would then be absurd. Dr. M. Mayer (pers. comm.) has observed discharging Trentepohlia sporangia,

from which the zooids are liberated posterior end first, and swim away without the intervention of a lag period after discharge. In Bryopsis, the gametes also become motile inside the sporangia, prior to their release in the laboratory.

There are two methods of zooid discharge in Enteromorpha intestinalis (1) Under cultural conditions small numbers of zoospores are discharged over a considerable period through a narrow tube. (2) In the natural environment, large numbers are released during short periods of time. It is the writer's view that the peculiar method of discharge in culture causes the actual period of release to extend over a longer time. Gayral (1960) also noted this but offered no explanation.

Variations from the normal form of zoospores and gametes. Burrows' (1958 a.) report appears to be the only published record of variation in zoospore shape. In this case the abnormalities were attributed to pre-treatment of the fertile thalli at 2°c overnight.

The writer found irrespective of whether release occurred in the natural environment or artificial culture a significant proportion of gametes or zoospores were always of abnormal shape; e.g. as a result of incomplete cleavage, two zoospores may be left joined to one another to various degrees. The number of abnormal zoospores was highest in regions which had already liberated most of their zooids.

Many of these forms (Figure 77) appeared to possess two groups of flagellae. The use of stains to confirm these observations was ruled out on the following grounds:

(1) All the stains tested except alkoholic iodine proved useless.

ENTEROMORPHA INTESTINALIS

Abnormal zoospores.

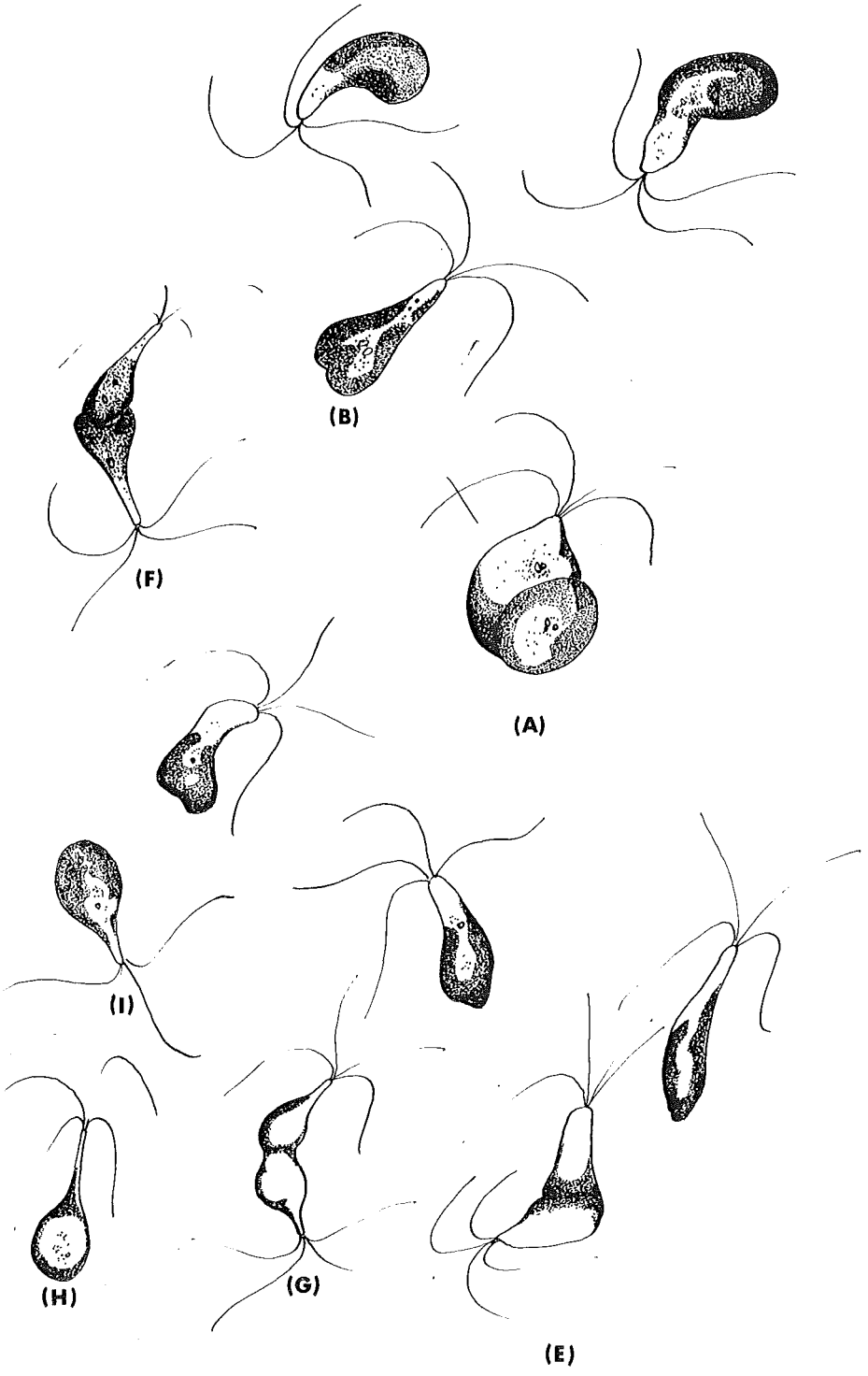


FIGURE 77

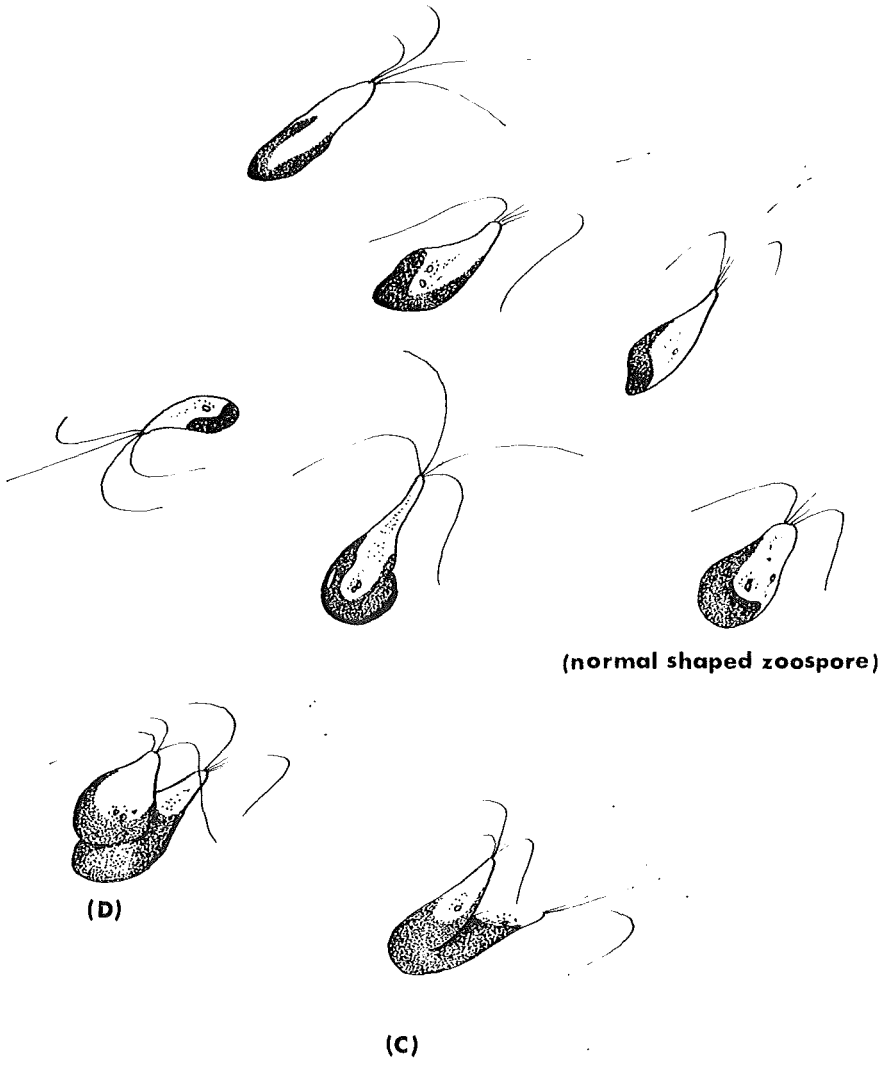


FIGURE 77 (cont.)

(2) The flagellae frequently abscissed in contact with iodine.

(3) It was extremely difficult to trace the comparatively few abnormal zoospores after they had mixed during staining with a large number of normal forms. In the active state, however, the abnormal zoospores and gametes are easy to trace because of their peculiar method of swimming.

Zoospores consisting of a large and small zooid joined laterally (Figure 77 A, B, C, D, E) usually had an uneven pattern of lateral swimming movement. Where the two were of equal size (Figure 77 C), the balance of swimming power between them was such that they could change their direction of swimming without any change in orientation. In these cases the "zoospore" did not revolve about its own longitudinal axis. Where two zoospores remained attached by their posterior ends the pattern of swimming was also very distinctive. There is no doubt that the flagellae at both ends were functional. The whole "zoospore" vibrated continuously, similar to normal zoospores during swimming, moved only a few μ in any one direction, and lacked any movement about its own axis. On one occasion the writer was fortunate enough to see one of these forms immediately after settling. Only three flagellae could be distinguished at either end.

The remaining source of evidence for the presence of two functional pairs of flagellae on many of these abnormal zoospores comes from observations of zooid release. As already noted, the discharge of zoospores posterior end first is restricted to sporangia of plants grown in artificial culture. When two zoospores remained attached by their postero-lateral walls (Figure 77 E, G), essentially two functional

FIGURE 78 - The writer has to date not sighted a published account of zooid morphology which describes the normal position of zoospore or gamete flagellae during swimming. While gametes are active they have one flagellum directed posterior the other anterior. When the direction of movement is changed, the flagellae are able to reverse their positions so that they are not determined as posterior or anterior by some intrinsic factor.

Zoospores have a pair of flagellae directed posterior and a pair directed anteriorly. The writer is not certain these can reverse their position relative to one another. It is possible that in both types of zooid the posterior flagellae provide the motivating force while the anterior directed flagellae control the direction of movement.

ENTEROMORPHA INTESTINALIS

gametes

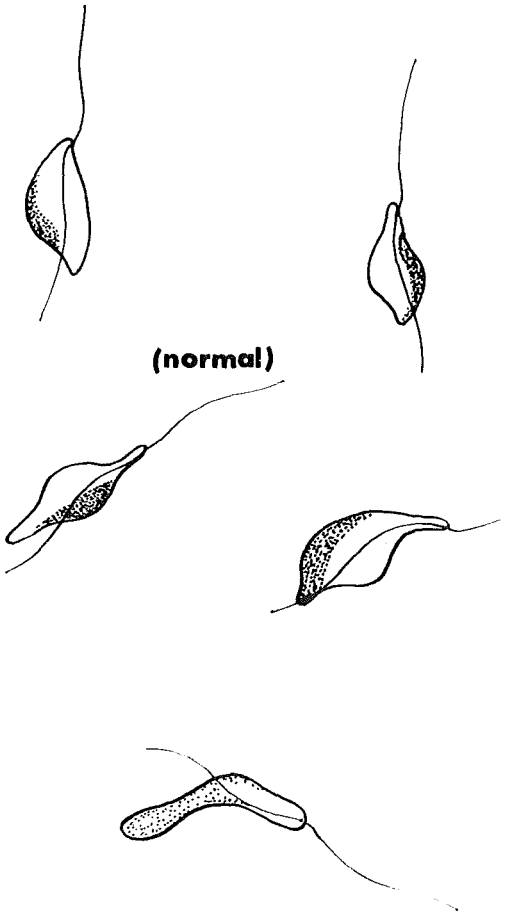


FIGURE 78

zoospores were formed. In these cases one partner alone appeared to find egress initially from the sporangium. The flagellae appeared to pass first through the exit canal - discharge therefore being anterior end first. At this stage a set of flagellae could frequently be seen at either end of the canal. This created a great problem for the attached partner still inside the sporangium, for there appeared to be no coordination of "effort" by the two sets of flagellae. This is evidenced by the considerable time these forms took for discharge, compared to normal zoospores. Eventually the partner inside the sporangium was pulled free by the continual tugging of the outer, usually more active partner. A diagramatic sequence for two such zooids joined only by their posterior ends is shown in Figure 76. Other types of zooid appeared to be released posterior end first.

A range of these abnormal zoospores is shown in Figure 77, and gametes in Figure 78. It may be concluded that;-

(1) the excessively long zoospores result from cleavage of the sporangial protoplast in one plane only,

(2) those zooids mutually attached at a 45^{o} or larger angle indicate that the cleavage planes may not always be orientated at right angles to each other as they are assumed to be in the literature,

(3) the form of the zoospores in Figure 76 indicates that right angle cleavage probably occurs in the minority of Enteromorpha intestinalis sporangia,

(4) attention has already been drawn to the variation in number of

cleavages between sporangia within the same small area of thallus. As a result, (a) there is a great divergence in the number of zoospores produced per sporangium, and (b) an even or an odd number of spores may result,

(5) the number of abnormal forms of gamete is considerably less than that of zoospores, and gametangial cleavage overall appears to be more regular.

The length of the motile period, phototaxis and gamete union. In most cases zoospores were found to settle on the first surface they contacted. Settlement began on an average, three minutes after release, with a maximum number settling seven minutes later. They rarely exhibited positive phototaxis possibly because the period of motility was too short.

There were indications that the zoospores of all plants did not behave similarly. On a few occasions they did not settle on the nearest surface, but swam to the brighter side of the container and collected at the water surface.

In all observed cases of gamete union there was no clumping. Apart from this, they behaved exactly as those of Ulva species described by Smith (1947).

In situ germination. The undischarged contents of gametangia appeared to be able to germinate satisfactorily in the laboratory and natural environment. However, 8 independent germination tubes were never produced, which means that either (1) some of the gametes aborted, (2) they were incompletely cleaved from one another, or (3) that fusion

of some occurred before germination. Kylin and Bliding (1963) found that Enteromorpha intestinalis was heterogamous, which would make such fusion unlikely. However, the writer could not always find a significant size difference between motile conjugants. In addition, the union of gametes while still in the gametangium has been recorded for several other algae e.g. Ulva (Schiller, 1907) Protosiphon botryoides, Hydrodictyon utriculatum (Klebs, 1896; Carter, 1926). Gametes from a single filament of Ulothrix zonata (Dodel, 1876) Gonium, Chlorogonium, Stephanosphaera and Vaucheria (Carter, 1926) may also conjugate amongst themselves. Although union of gametes within a single sporangium is known, the possible abortion of a number within each sporangium before germination, however unlikely, cannot be ruled out.

Careful observations of undischarged zoosporangia, maintained in artificial culture, showed that the zoospores in some appeared to fuse before germinating. In other cases two large bodies within a single sporangium each produced a uniseriate filament. These could have originated by the fusion of the undischarged zoospores in two equal groups, or from a zoosporangium which cleaved only once. Frequently a single zoospore, remaining after the rest had been discharged, would germinate (Figure 79).

It first elongates into a narrow rhizoid-like tube extending through the hole in the wall. At this stage the germling has an expanded 'foot' inside the sporangium, formed by an increase in size of the parent zoospore. The chloroplast appears to pass into the tube along one wall, and frequently the first cross wall leaves the foot almost colourless. Subsequent growth is restricted to the filament outside the sporangium.

IN SITU GERMINATION OF AN UNRELEASED ENTEROMORPHA INTESTINALIS ZOOSPORE

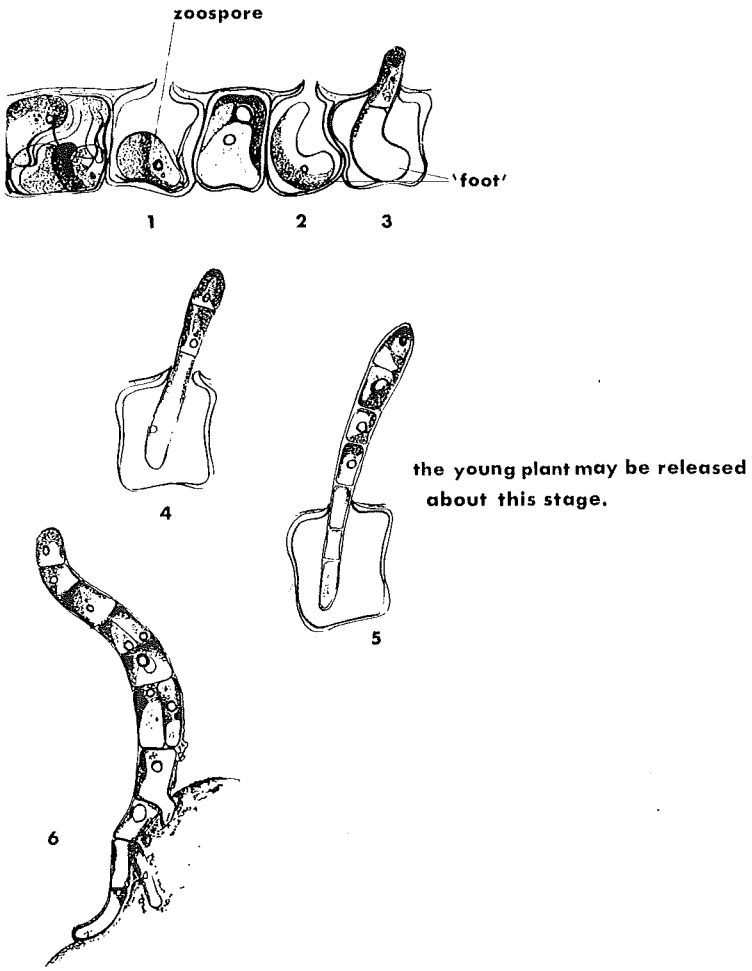


FIGURE 79

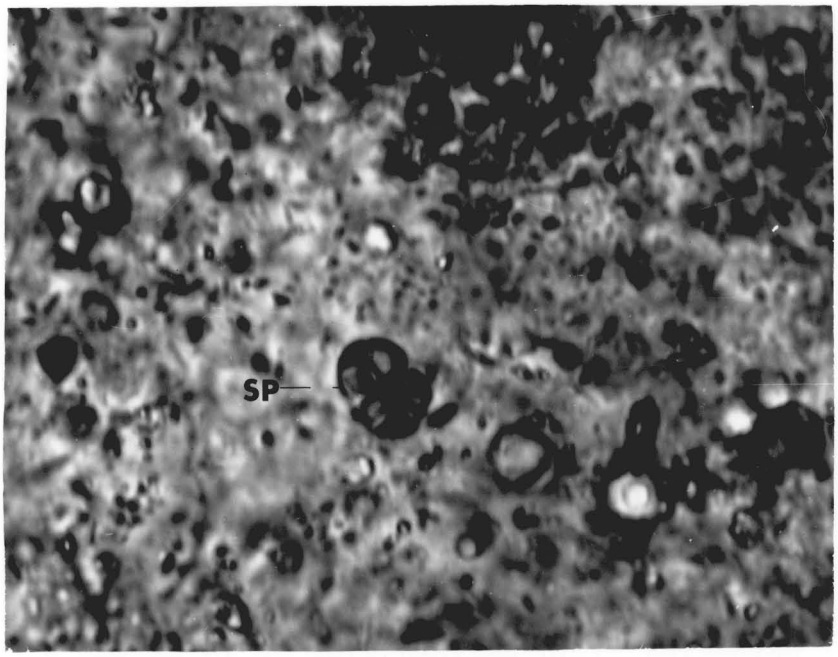


FIGURE 80 - An undischarged sporangium (SP) in an Enteromorpha plant collected from Woodpecker Bay, West Coast.

FIGURE 81 - In situ germination of an undischarged sporangium of a plant collected from Woodpecker Bay, West Coast.

It seems likely that at about the 6 celled stage, when the foot has disappeared, the sporeling would present sufficient resistance to water movement to be removed from the thallus. Further significant growth would only occur when the young plant has settled because:-

(1) It was demonstrated experimentally that strong sunlight brings about modification to the chloroplast, and is therefore probably mildly injurious.

(2) Pelagic plants do not appear to be able to form a great number of normal upright thalli. This aspect is discussed in more detail in a subsequent section.

Figure 80 shows a region of an Enteromorpha plant collected from the supralittoral zone, Woodpecker Bay, West Coast on 13.5.65 with an undischarged sporangium, and Figure 81 one in which in situ germination has commenced.

It is interesting to note here the similarity between certain aspects of zooid release under cultural conditions, and in situ germination. When undischarged zoospores germinate, development always commences at the posterior end, the same as in discharged zooids, in which the anterior end forms the attachment organs. When zoospores are discharged from the sporangium under cultural conditions, the tube by which they gain egress is always formed from the posterior end. It would appear that these characteristics are manifestations of a strong polarity.

Another type of reproductive body, which gives rise to new plants by in situ germination was also found during this series of investigations.

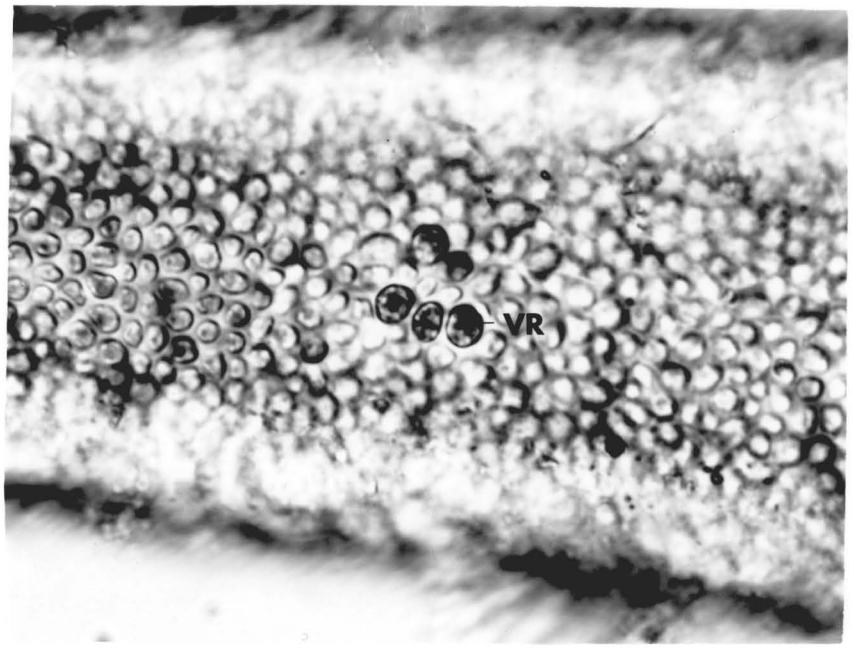


FIGURE 82 - The formation of vegetative cells specialised for reproduction (VR) near the base of an Enteromorpha plant.

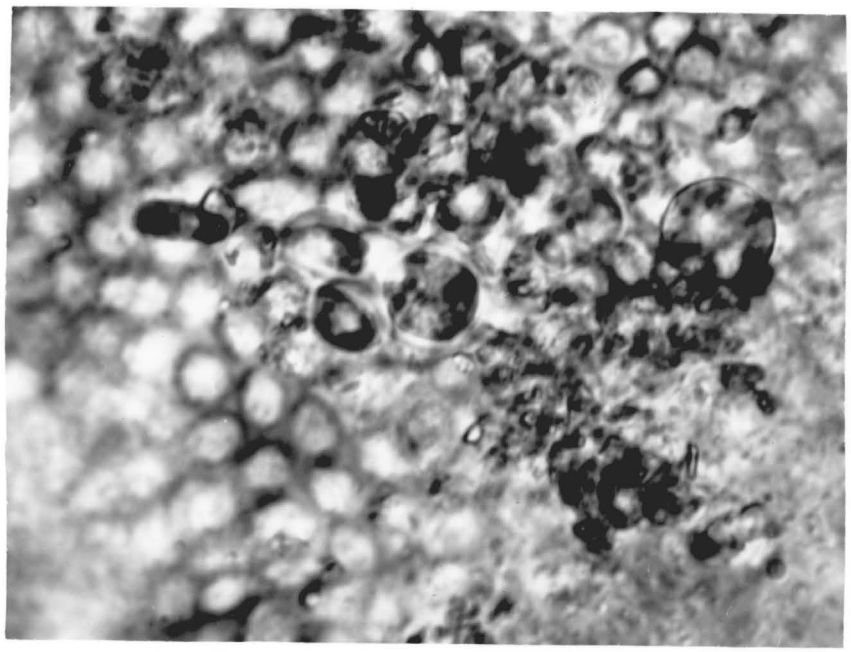


FIGURE 83 - In situ germination of one of the above reproductive bodies.

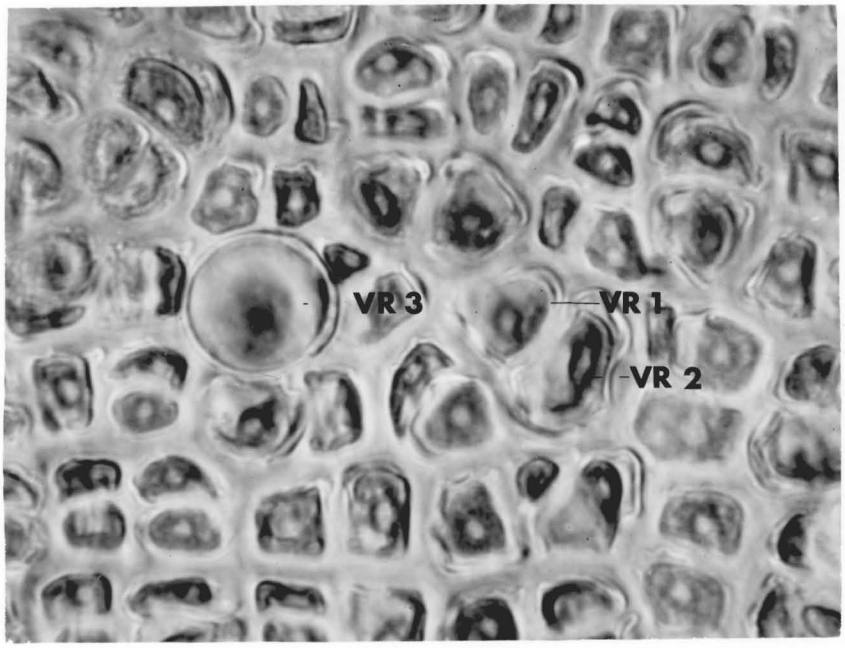


FIGURE 84 - Stages in the formation of cells specialised for reproduction by in situ germination (VR 1 - 3) in the fertile region of an Enteromorpha intestinalis plant.

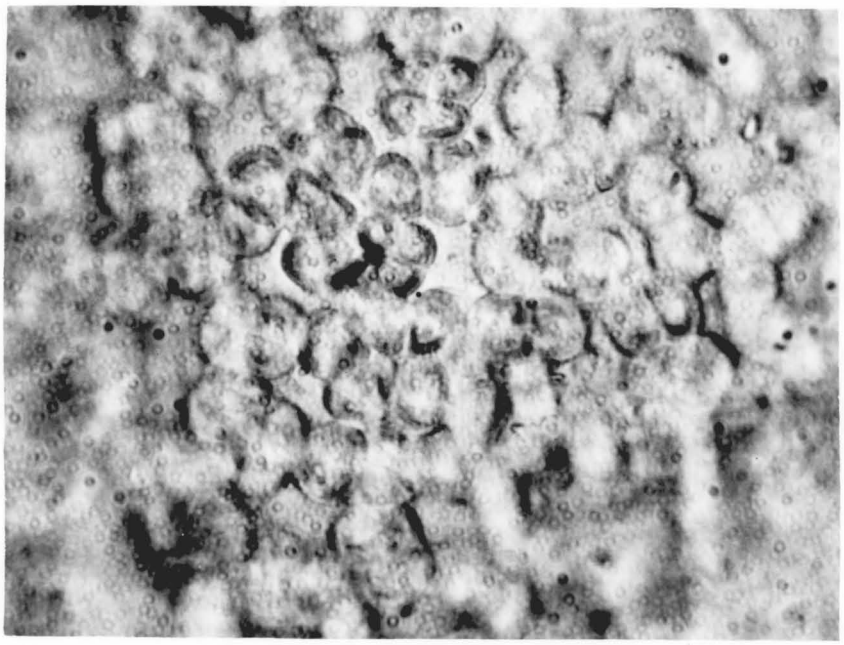


FIGURE 85 - An anastomosing network of filaments formed by in situ germination of undischarged Enteromorpha intestinalis zoospores.

These were large vegetative cells, which only differed from normal cells in their large size and dark green chloroplast. Where these form in actively growing vegetative regions of thallus (Figure 82), their distinction from sporangia or gametangia is clear. They may even germinate before the surrounding tissue becomes fertile (Figure 83). However, in zoospore producing plants of Enteromorpha intestinalis stages in the formation of these cells may be seen in regions where most of the cells, are dying or forming sporangia (Figure 84). Their distinction from other types of reproductive body in time and space is therefore not always so distinct.

Finally, it is difficult to distinguish this type of reproductive body from a germinating zoosporangium in which the zooids have almost completely fused. However, they appear to be similar to structures described by Provasoli (1958 a.), Dangeard (1957 b.), and Lovlie (1964). It is almost certain that these authors were not aware of the narrow distinction between this range of reproductive phenomena.

During the course of culturing a population through several generations, the writer was able to compare the morphology and viability of Enteromorpha intestinalis plants which had not been released from the parent thallus, following in situ germination, with those which had been. Both exhibited the same morphological features, and produced normal quadriflagellate zoospores at the same time.

On 6.6.66 a number of plants of the type characteristic of the summer generation were collected from point B (Figure 9) in the Motunau River. The regions which had discharged their zooids exhibited a number of small pale red / brown blotches. Microscopic examination revealed that

these were caused by an anastomosing network of large rhizoid-like filaments, with similarly coloured plastids and a large number of granules (Figure 85). They appeared to originate by in situ germination of zoosporangia, or enlarged vegetative cells. A considerable amount of fusion had apparently occurred between the filaments of this formless mass. Cultural experiments with the same generation a year before showed that single undischarged zoospores frequently developed only a colourless rhizoid system by in situ germination. One main branch would be produced by each zoospore, this would grow between the cells inside the parent thallus, sending out short lateral extensions morphologically rather like fungal haustoria into the intercellular spaces. These appear similar to branched rhizoids described by Carter (1926) from the stipes and holdfasts of Ulva species, whereas the larger masses of brown pigmented filaments growing upon the thallus do not appear to have been previously recorded.

The implications of in situ germination. In the discussion of the ratio of gametophyte to sporophyte plants, two explanations were suggested for the existence of zoospores in a predominantly gamete producing winter generation:

Either (1) there was a small number of zoospore producing plants growing during the winter,

or (2) gametes and zoospores could be produced, in a few cases, on the same thallus, but released at different times.

If, as the writer believes, undischarged zoospores fuse before in situ germination, it is probable that they give rise to a winter zoospore-producing generation. Conversely, if unfused gametes germinated

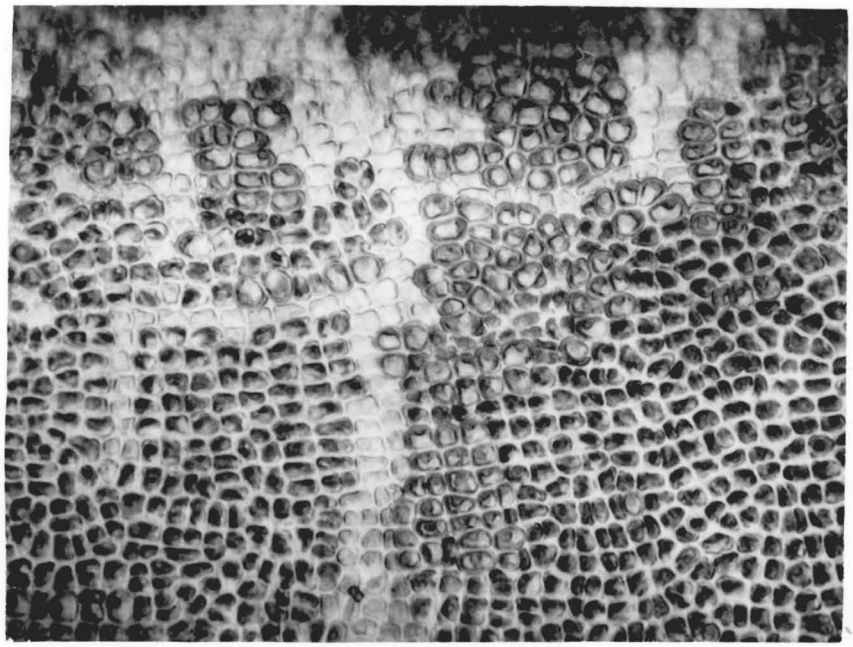


FIGURE 86 - The mosaic distribution of zoosporangia in Enteromorpha intestinalis.

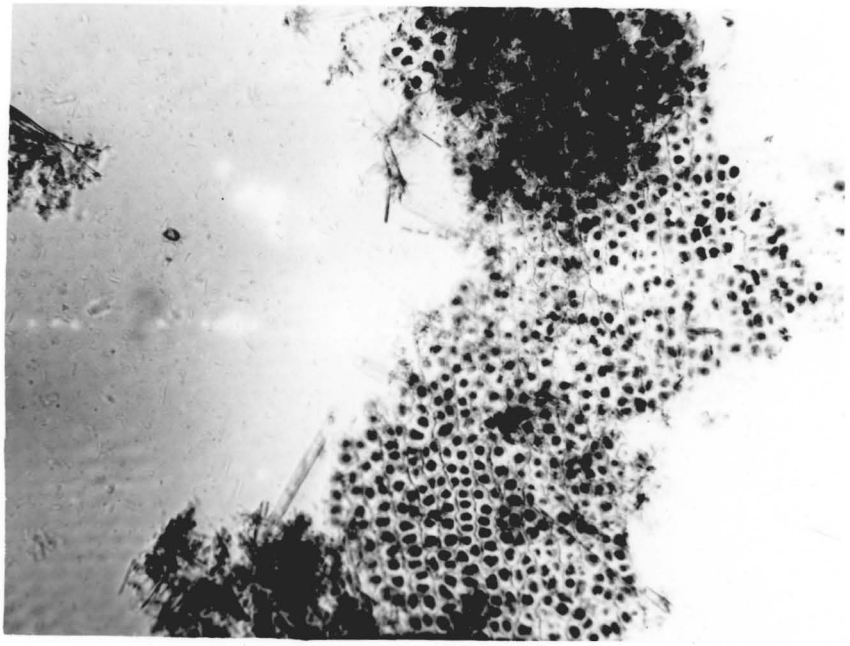


FIGURE 87 - Pieces of vegetative tissue freed from fertile regions of Enteromorpha intestinalis by the mosaic distribution of zoosporangia.

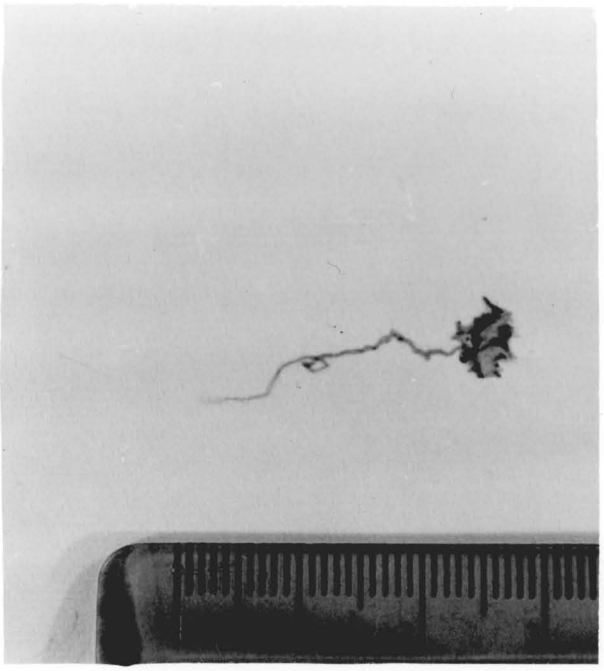


FIGURE 88 - An Enteromorpha plant attached to a piece of vegetative tissue, collected from the supralittoral zone, Bluff.

in situ (apomiktosis) summer gamete producing plants might result. If the writer's interpretations were correct these processes would be yet another expression of the extreme plasticity of Enteromorpha. Not only may gametes act as zoospores but zoospores may also act to a certain extent as gametes.

"When Ulva is growing in quiet waters of estuaries, it may multiply vegetatively by growth of fragments accidentally detached from a thallus", (Smith, 1964). "This .... may lead to the formation of loose lying communities", (Fritsch, 1961). Both Carter (1932) and Chapman (1956) have observed the formation of loose lying Enteromorpha communities. However, the writer feels that vegetative reproduction by fragmentation plays a far more important role in the seasonality of Enteromorpha intestinalis, and probably other species, than these reports suggest.

By the end of the summer at Motunau, many plants have been completely consumed by production of swarmers. However, some are not completely consumed in this way, as swarmer production occurs in a mosaic pattern basipetally in the thallus, isolating irregular areas of tissue (Figure 86). These are set free by breaks along the former reproductive areas (Figure 87) and float about in the river for a time. On 6.6.66 the writer collected approximately 1 lb of these fragments, which had been cast into small mounds by the action of wind and tide.

Eventually they appear to sink, and give rise, probably by in situ germination, to new plants. During the winter of 1965, many of the fragments subtending mature plants were found attached to the rocks by a covering of mud. One similar plant (Figure 88) was found at Bluff.

Summary: The Processes Leading up to Zygote and Zoospore Formation.

(1) The Motunau River population of this species exhibits a distinct seasonality. A winter dominantly gamete producing generation alternates with a summer predominantly zoospore producing generation.

(2) The exact ratio of gametophytes to sporophytes in each generation is dependent upon -

(a) the number of plants which possess regions or cells capable of producing the type of zooid characteristic of the other generation,

and (b) the number of solely zoospore producing plants growing in a predominantly gamete producing generation, and vice versa.

(3) A previously unrecorded range of zooid shape was found. This, together with observations of sporangial cleavage, support the conclusion that the processes of sporogenesis, and to a lesser extent gametogenesis, are not nearly as regular as existing records suggest.

(4) No colour differences between fertile and vegetative gametophyte plants could be found. The colour of fertile regions of sporophyte plants grown in culture does not agree with existing records.

(5) Several methods for initiating zooid release detailed in the literature were unsuccessfully tested on this species. At certain times of the year, a variety of methods were found effective, but the range of conditions under which zooid release proceeded indicated that any precision in release stimuli, advocated in the

literature, is entirely unnecessary. Without several years of observation, the writer could not be certain whether the periods when sporangial discharge stimuli were effective, were determined by inherent periodicity.

(6) Two methods of zoospore discharge were found, one restricted to artificial cultural, the other to the natural environment. The cultural method has not been recorded before.

(7) The following types of cell may germinate 'in situ'; ? fused gametes, unfused and fused zoospores, normal size and enlarged vegetative cells. As a result, normal plants and rhizoid-like growths of various types may be produced. Pieces of vegetative tissue, isolated from the parent thallus by zooid formation, appear to play a significant role in the periodicity of this species. Such a range of reproductive behaviour has not been previously described to the writer's knowledge.

EMBRYOLOGICAL VARIATION IN THE GENUS ENTEROMORPHA

Literature.

According to the accepted description of embryology for this Genus, zoospores and zygotes develop in the following way. The zooid settles and divides into two cells, the lower basal one giving rise to the holdfast, the upper one to the upright system (Dangeard, 1961; Smith, 1964, P.63; Fritsch, 1961, P.217). The early stages of this sequence as illustrated in Figure 90 from Baudrimont (1960) are the same in Ulva, Enteromorpha and Letterstedtia (Pocock, 1959).

However, over recent years there have been reports that this sequence may vary to some extent. Provasoli (1958) reported the existence of sporelings in Ulva, which possessed no recognisable pattern of development. Dangeard (1960) found the following types of sporeling in Enteromorpha Linza, in addition to those developing normally.

(1) Sporelings in which the development of the upright filament was retarded because the first formed filament cells remained applied to the prostrate system (L.M. Figure 91).

(2) Young plants in which two filaments formed simultaneously from a very reduced base (H. Figure 91).

Baudrimont (1960) also working on Enteromorpha Linza found that (1) sporelings with no organised pattern of development (Figure 92 c.) and (2) those in which the prostrate system developed first, occurred regularly in several culture solutions, including those of Kylin (1941) and Schreiber (1927).

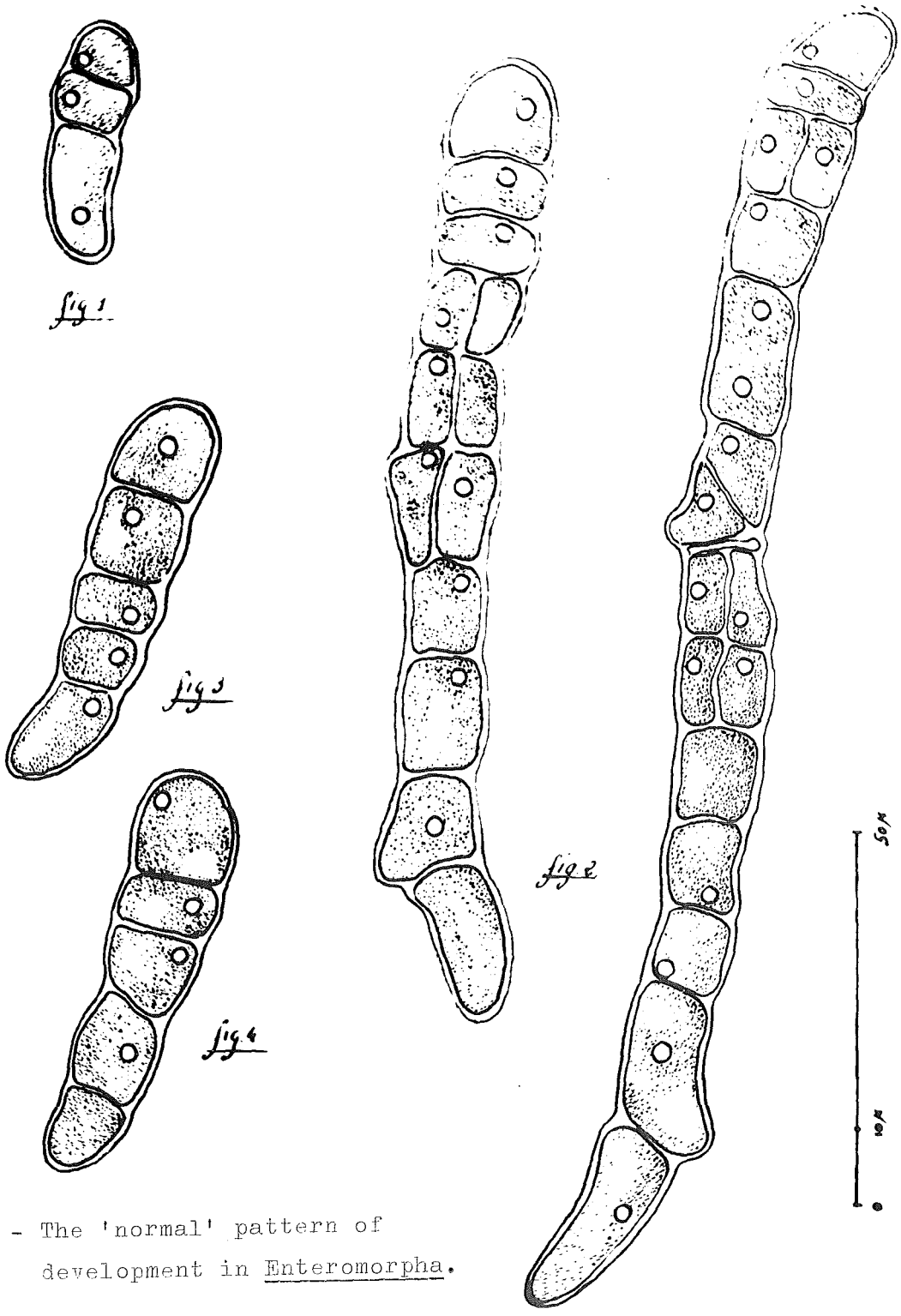


FIGURE 90 - The 'normal' pattern of development in Enteromorpha.

PLANCHE II

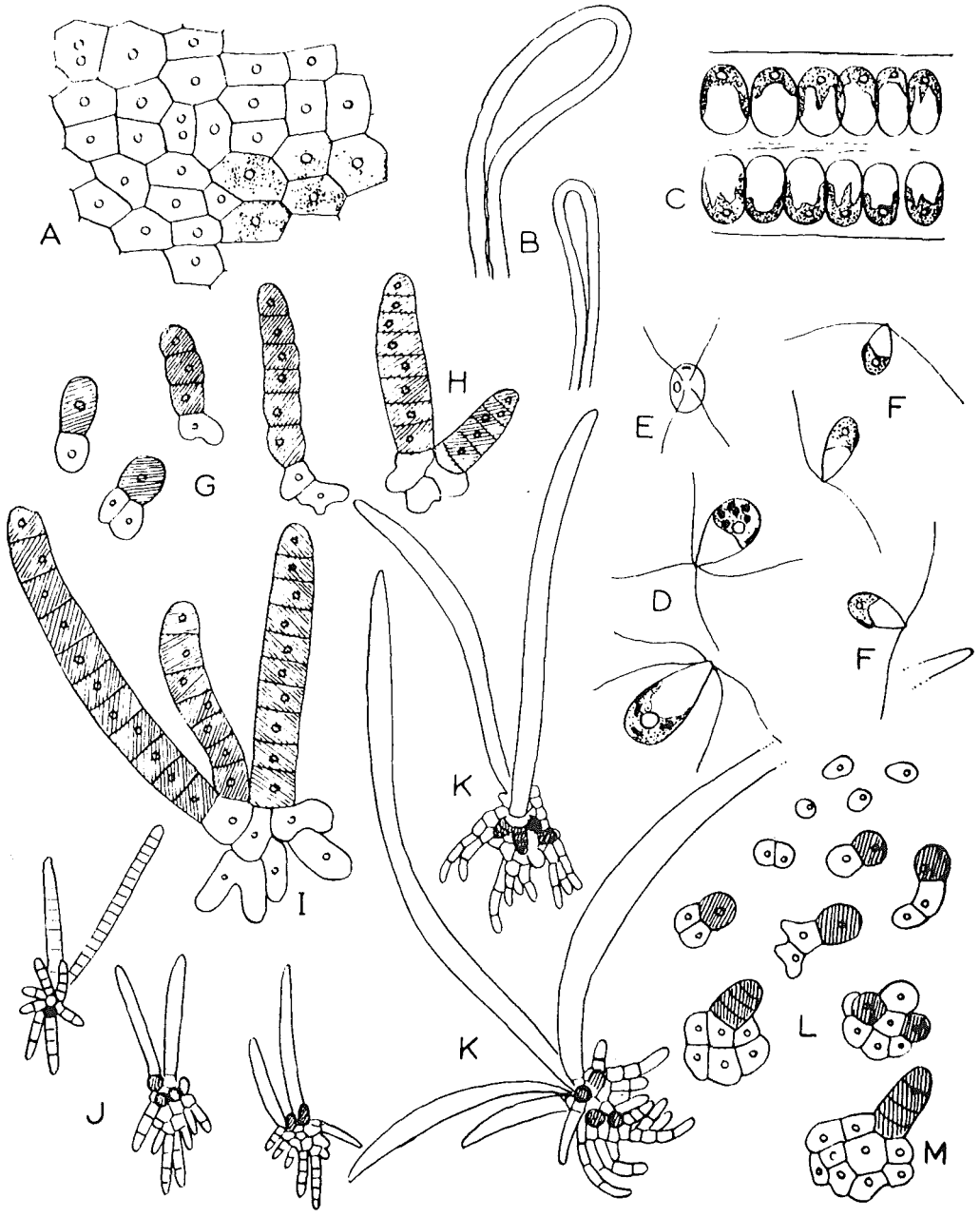


PLANCHE I

Enteromorpha Linza (L.) J. Ag.

FIGURE 91 - The embryological variations found by (Dangeard, 1960) in E. Linza.

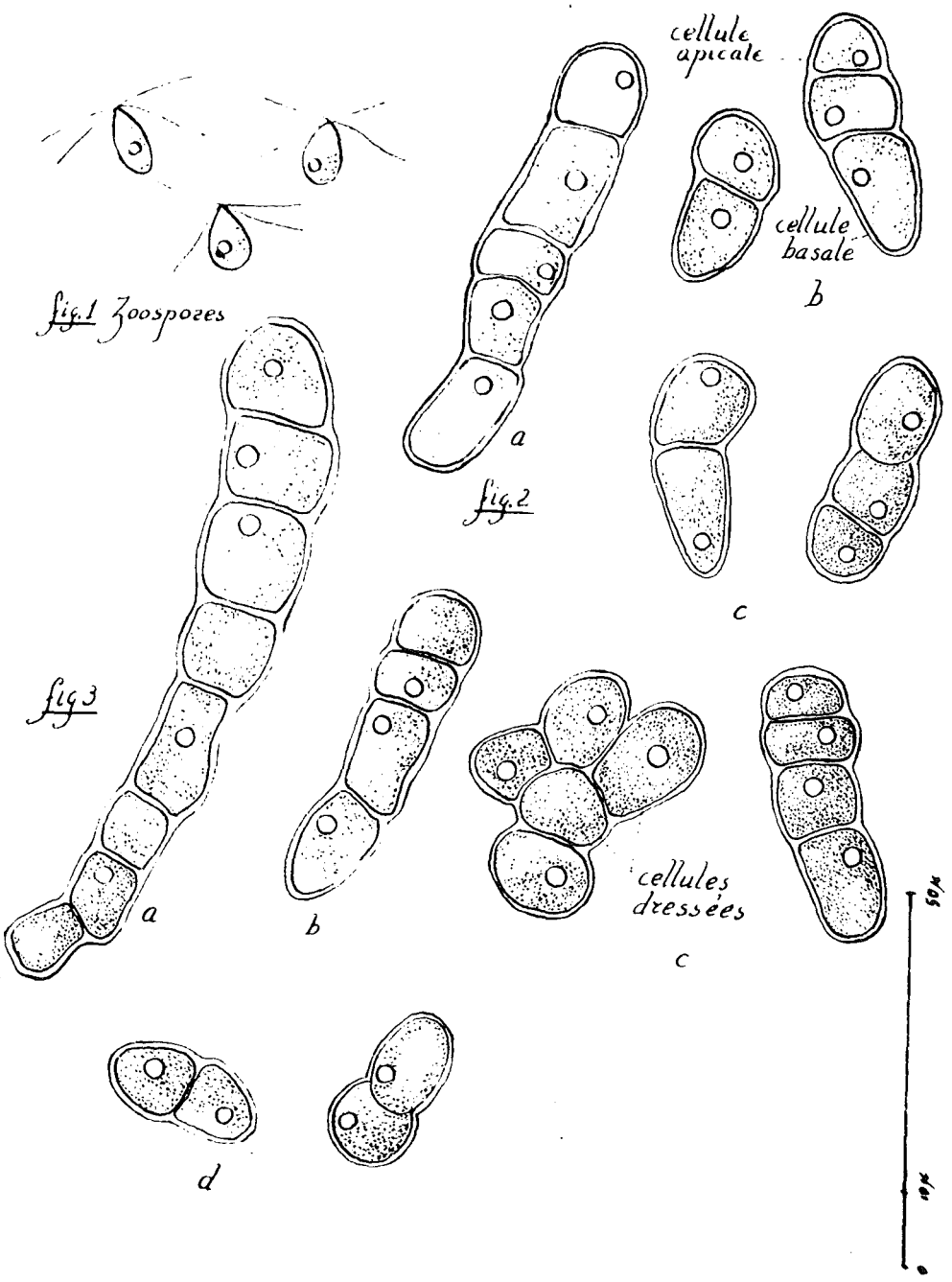


PLANCHE I

FIGURE 92 - The early stages of zoospore germination in Enteromorpha Linza from Baudrimont (1960).

Pocock (1959) found a variation in the development of Letterstedtia insignis Areschoug zygotes. As a similar range could not be found in sporelings of comparable age growing in the natural environment, it was concluded that the cultural variations were abnormalities.

However, within the broader framework of the Ulvales (Bliding, 1963) there are well documented cases of variations from this classical embryological pattern. The Genus Blidingia Kylin was established to include those members of the Ulvales which developed their thalli from a prostrate distromatic holdfast. Kornmann (1956) showed the existence of two types of embryology in the related Percursaria Bory 1823. The upright filaments mostly arise from the margins of a one-layered disc, but not infrequently there was a direct development similar to Figure 90. This has been confirmed by Bliding (1963). A horizontal disc also forms first in Enteromorpha hendayensis, (Dangeard and Parriaud, 1960). From this one cell differentiates as an apical cell and forms a single uniseriate filament. Later, several other cells may differentiate into apical cells each producing a separate filament. Føyn (1961, 1962) has recorded several mutants in Ulva mutabilus, while more recently Kornmann (1965) appears to have revealed a comparable situation in Ulothrix.

From this survey it is evident that there are two types of variation from the normal embryology.

(1) Small variations probably not under strict genetic control.

(2) Variation under rigid genetic control.

It is possible that the variations which occurred in the cultures

of Provasoli (1958), Pocock (1959), Baudrimont (1960), and Dangeard (1960) were not under rigid genetic control, whereas those found by the following were: Kornmann (1956), Dangeard and Parriaud (1960), Føyn (1961, 1962), Kornmann (1965). When the present investigations were begun, the writer did not have the benefit of this literature survey. In fact many of the papers were not finally obtained until the draft was being written. The only facts which were known to the writer were:

(1) that a range of variation from the normal embryological pattern occurred in the first cultures established for this study, and

(2) that this did not appear similar to the genetically controlled variation cited above which was used as a taxonomic criterion.

In view of the range of variation recorded for most higher plant taxonomic characters and the preliminary observations of the present study, it was postulated that a range of variation was a normal part of development in the Genus Enteromorpha. The writer completely disclaims any influence whatsoever from published accounts of variation on the initiation of these experiments. It was believed at that time that this was the first specific investigation into non-genetically determined variation, and in view of the literature survey this is probably still correct.

Embryological Variation of an Enteromorpha intestinalis population at Motunau.

On 15.3.65 fertile thalli of the summer generation of E. intestinalis were collected from Motunau. As these released only a few zoospores, the fertile thalli were placed between a slide and No. 1 cover slip, in order to retain the few resultant sporelings together as much as possible.

The cultures were maintained in a peat Erdschreiber solution changed every 2 days, and illuminated by two 80 watt fluorescent tubes 6" away.

After 5 weeks' growth the following observations were made. Many sporelings remained unbranched, and although varying greatly in the relative speed of their development, otherwise conformed to the usual pattern of development. However, the following variations were recorded in addition to this type.

(a) Various forms of branched plant. In some, longitudinal division commenced at the tip, where one of the subapical derivatives simply divided again in the same plane to initiate a branch (Figure 93). In other plants a cell in the median region of the uniseriate filament divided longitudinally and initiated a branch (Figure 94).

(b) The sporelings which appeared to possess little or no organised pattern of development were by far the most striking. Some had developed normal blade cells and rhizoids at opposing ends of the sporeling for a period, and then lost this polarity.

As a result, rhizoids also developed at the tip of the blade (Figure 95). Others possessed no organisation from the initiation of their growth (Figure 96).

This fact alone suggested to the writer that some zooids might develop abnormally as a reflection of an inherent abnormality. In order to confirm this and determine whether similar variants to those in Enteromorpha intestinalis could be expected in other populations raised in artificial culture, the following experiment was carried out.

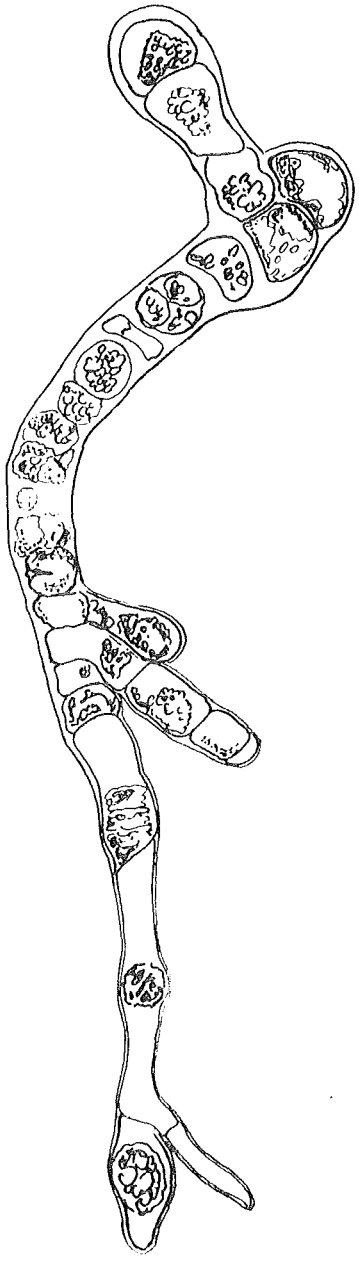


FIGURE 93 - Branch formation by a restriction of longitudinal divisions to a single subapical cell and its derivatives.

FIGURE 94 - Branch formation by a restriction of longitudinal cell divisions, to an intercalary cell and its derivatives.

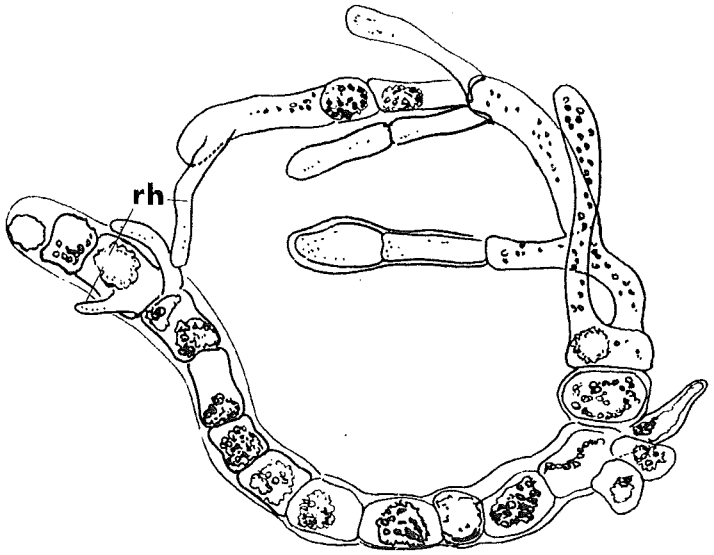


FIGURE 95 - A young plant of Enteromorpha intestinalis which has developed rhizoidal cells (rh) at both ends of the thallus.

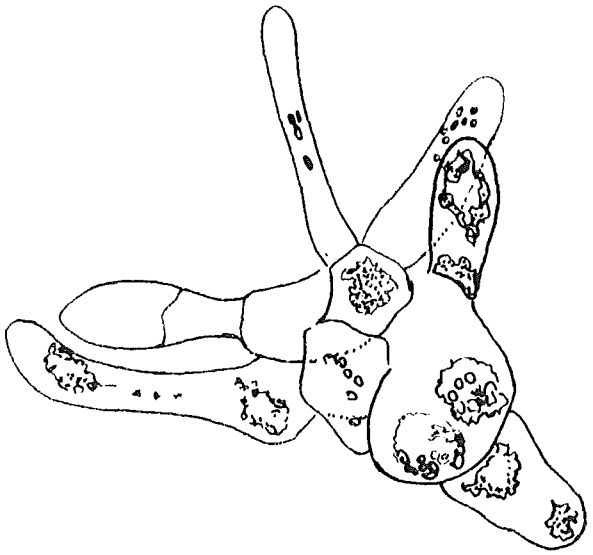


FIGURE 96 - A 'plant' which developed from a zoospore of Enteromorpha intestinalis, lacking any organised pattern of growth.

In conformity with the aim of incorporating as many populations from as widely separated geographic areas as possible into this study, the fertile material was collected from the Supralittoral zone, Woodpecker Bay, West Coast on 13.5.65.

The Variations of Growth Pattern of the Woodpecker Bay Population.

Zooid release occurred on 13.5.65. The following day the swarmers were transferred to Petrie dishes containing peat Erdschreiber solution and placed under the same bank of lights as the Motunau population. The following range of development was photographically recorded five weeks later. For convenience, the variation in uniseriate plants is considered separately.

Variation of sporelings at the uniseriate or comparable stage of development. All the variations recognised were produced by a loss of or variation in the normal orientation of cell division. Thus a cell in any position on the filament could divide in any plane.

A normal longitudinal division occurring in a median cell could initiate branch formation (Figure 97). However, this longitudinal division was frequently so oriented that it formed two unequal cells (Figure 98) the subsequent growth of which forced the smaller partner to occupy a lateral position on the filament, (Figure 99). This could also occur in the subapical cell (Figure 100) or several of the terminal cells (Figure 101) causing the filament to lose its orientation.

The apical and occasionally the subapical cell of a filament were able to divide. This resulted in 2, 4, or even 8 cells at the tip of a single tier of cells. Figure 102 shows the expansion of the apical

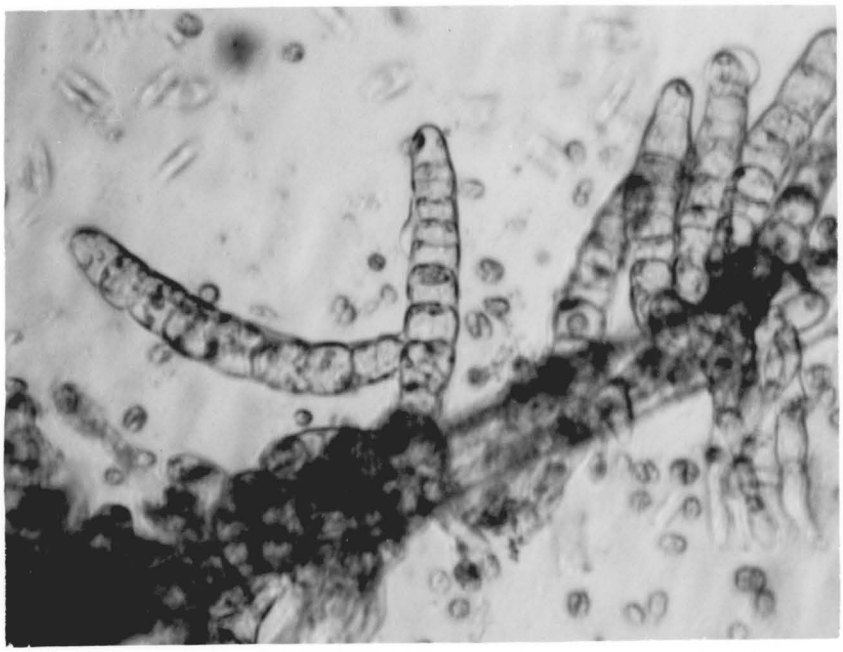


FIGURE 97 - Branch formation by longitudinal division of an intercalary cell. x400

FIGURE 98 - A filament in which longitudinal division has formed two cells of considerably different size: (1) small, (2) large. x600

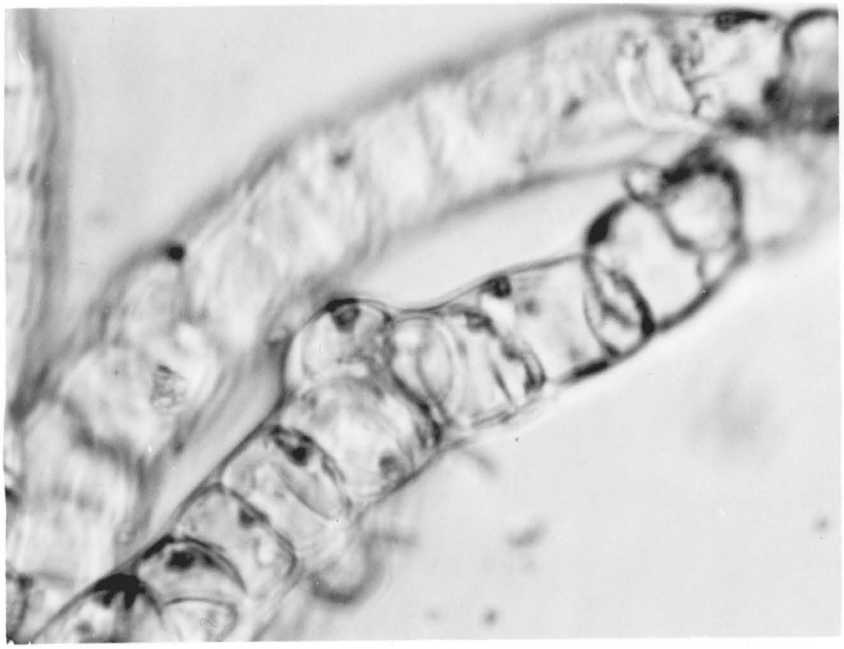


FIGURE 99 - A filament showing the complete displacement of a small cell produced by longitudinal division, from the main series. x600

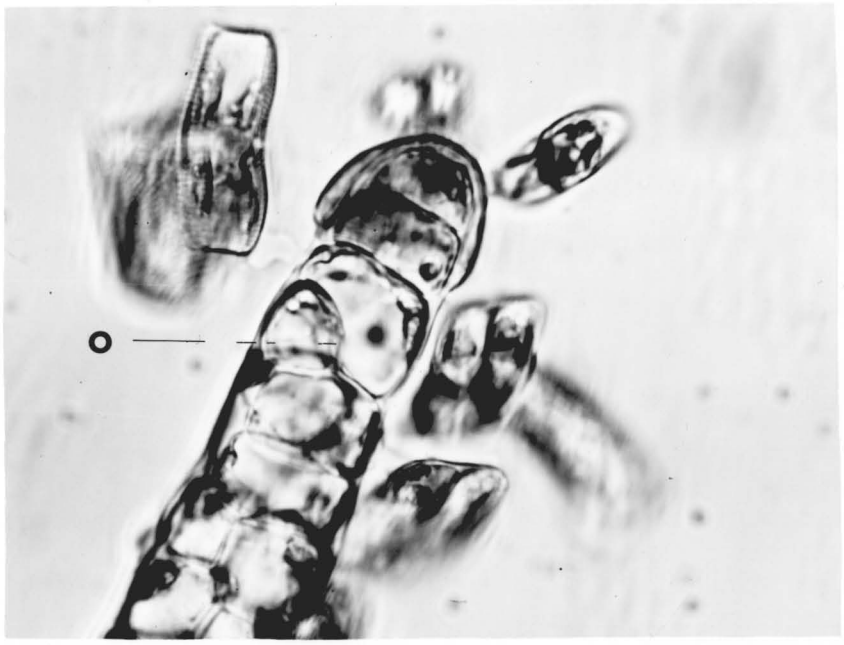


FIGURE 100 - Showing an oblique cell division (O) in a subapical cell. x600

cell prior to division, Figure 103 two cells resulting from such a division, oriented at an angle to the longitudinal axis, and Figure 104 two cells produced by division parallel to this axis. These two may grow and eventually each give rise to an independent filament, Figure 105. Less often the apical cell was divided into four by two vertical divisions, (Figure 106). Occasionally this would even occur to the subapical cell also (Figure 107).

A further line of variation, including alterations in the orientation of both intercalary and apical cell division could be distinguished (Figure 108). The culmination of this line were the plants which completely lacked any organised pattern of development (Figure 109).

The sporeling in Figure 108 has an abnormally large median cell in the filament, compared to the others. Similar cells may also occur in a multiseriate thallus of any size. It is these cells which the writer wishes to be ignored when character limits such as cell size are established for that species.

Variation of Sporelings at the Multiseriate Stage of Development. Cells in median regions of the thallus divided unequally, the smaller portion frequently being squeezed out of the series into a lateral position on the thallus.

One of the most interesting observations made was that the holdfast of these more mature plants was not composed entirely of rhizoids (Figure 110). In fact, cells closely resembling normal blade cells and rhizoids composed the holdfast. Further, both could be formed in the same cell series (Figure 111). On the basis of this evidence the following may be concluded.

FIGURE 101 - A filament in which several terminal cells have lost the regular orientation of their division planes. x600

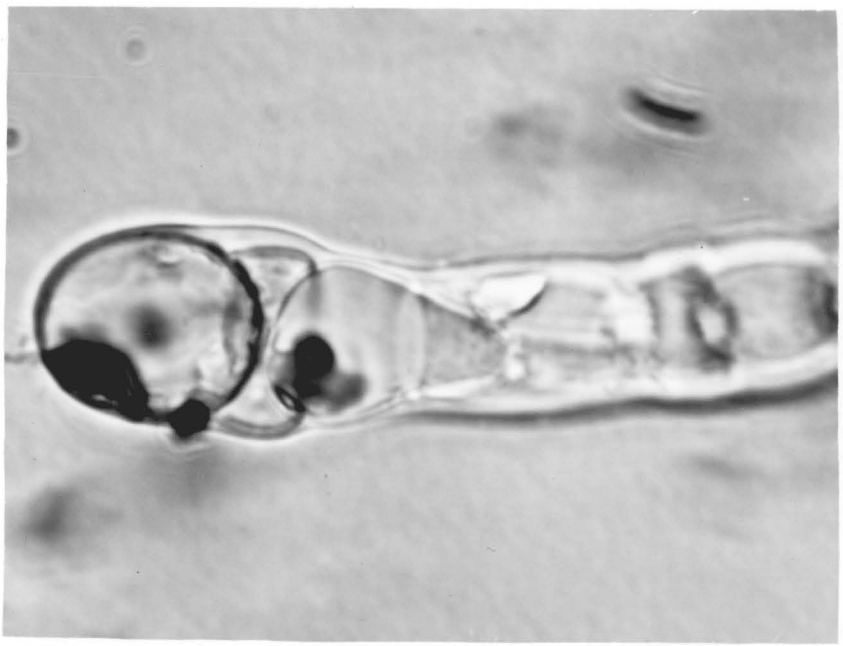


FIGURE 102 - Expansion of the apical cell prior to division. x600

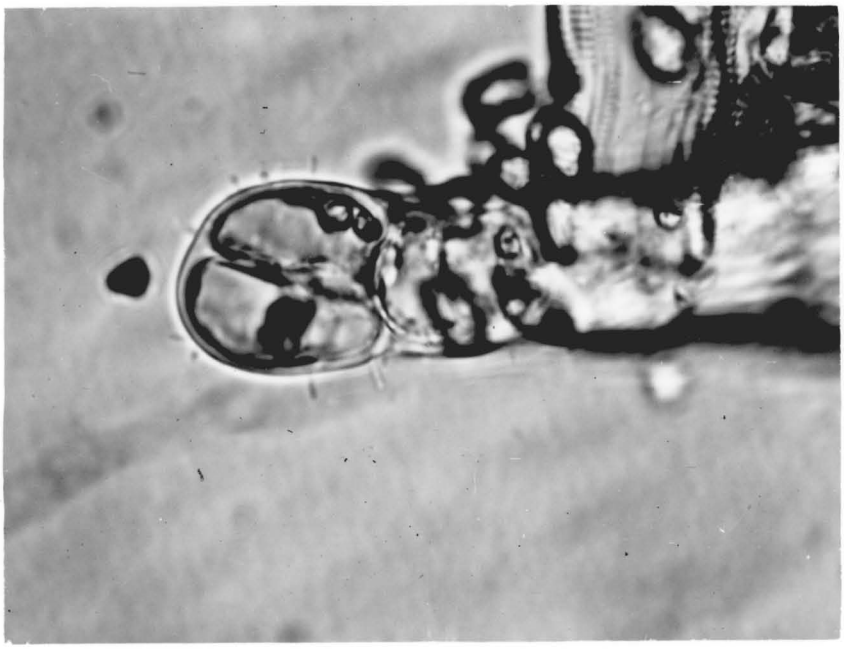


FIGURE 103 - An apical cell after one dichotomous division. x600

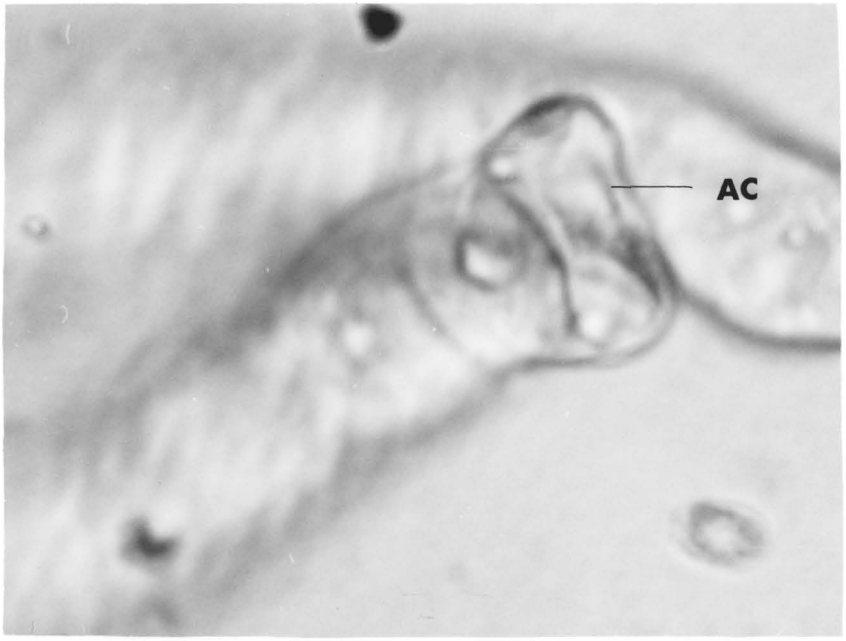


FIGURE 104 - An apical cell (AC) dividing along a plane parallel to the longitudinal axis of the filament. x600

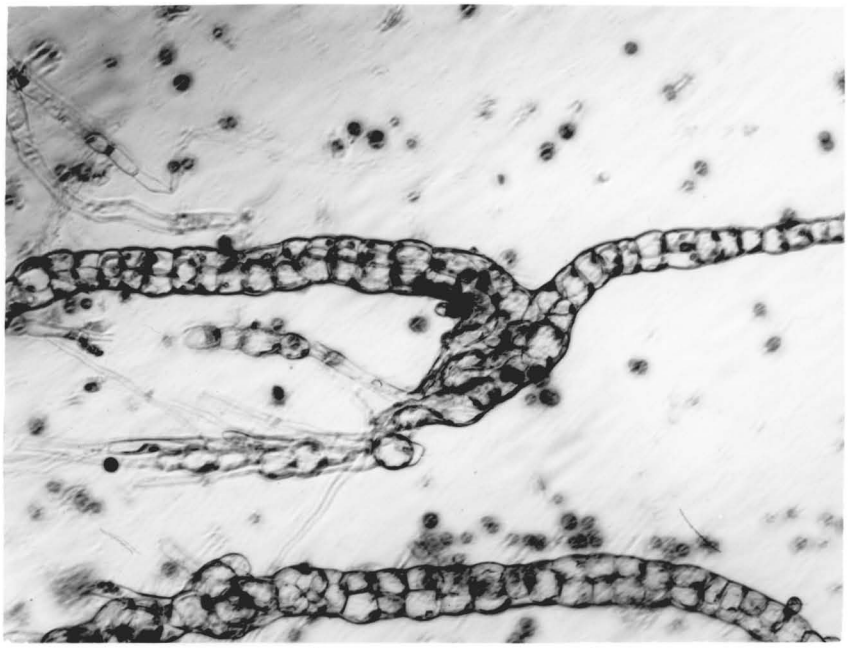


FIGURE 105 - A dichotomously branched plant. x400

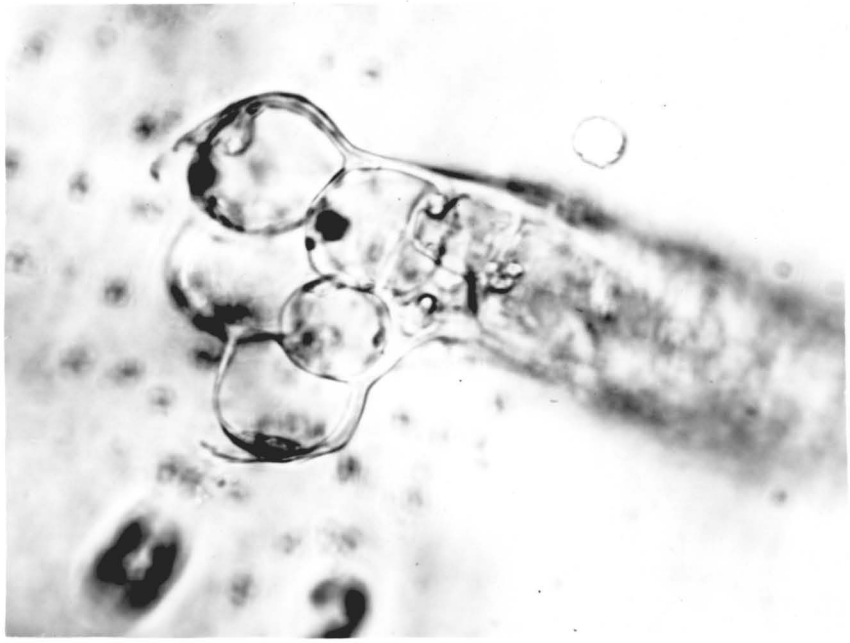


FIGURE 106 - An apical cell which has divided into four by two divisions orientated at right angles to one another. x600

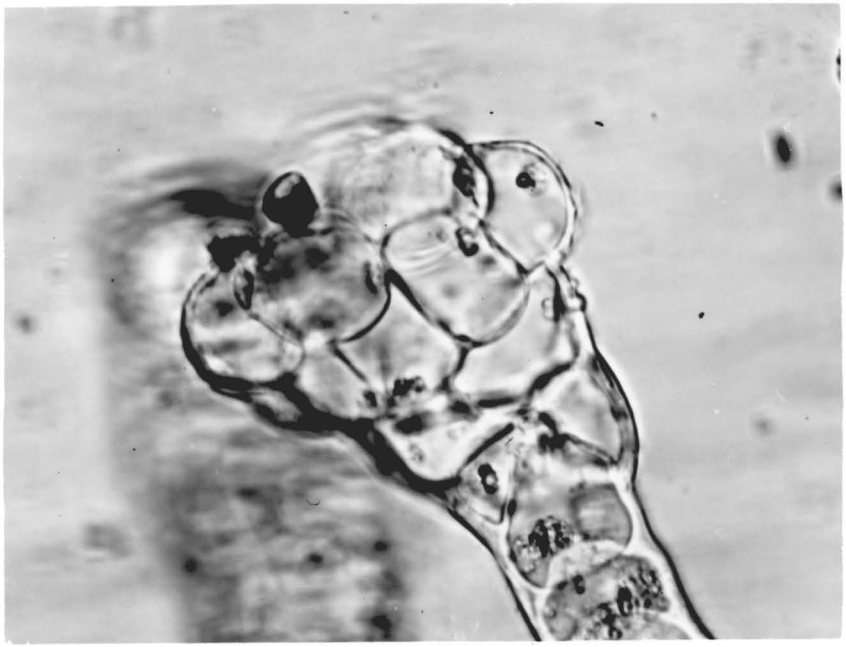


FIGURE 107 - A filament in which the apical and subapical cells have divided into four. x600

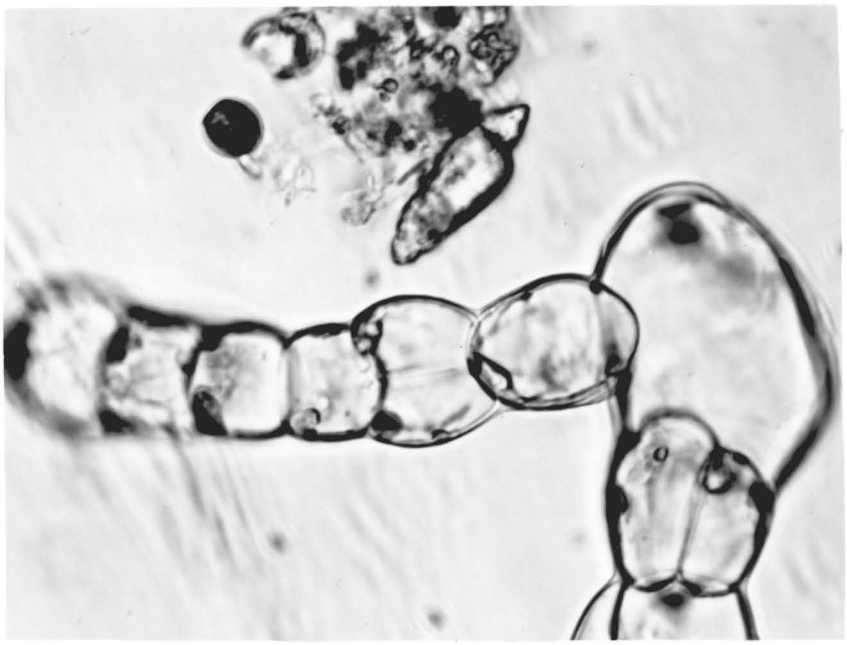


FIGURE 108 - A filament in which the orientation of apical and subapical cell division is irregular. x600

FIGURE 109 - A 'plant' which lacks an organised pattern of growth. x600

FIGURE 110 - A detailed photograph of a holdfast showing cells closely resembling vegetative cells (V) and rhizoids (R). x600

(1) There is a delicate balance between the two cell types, and

(2) to maintain the dominance of rhizoids in holdfasts, so frequently alluded to in descriptions of Enteromorpha ontogeny, a stimulus appears to be needed which tips this equilibrium in favour of rhizoid development. In the Woodpecker Bay population, the balance between the two cell types is so delicate that rhizoids and normal blade cells occur in the same filament.

A range of variation comparable to that of the Woodpecker Bay population was found in sporelings from a Little Papanui population. (The fertile material for this experiment was supplied by Dr. E.J. Batham).

Variation of Embryology in an Enteromorpha population from Little Papanui, Otago Harbour.

In this population a large number of plants exhibited some variation of the normal developmental sequence (Figure 90). These variations could be divided into two distinct patterns. In some uniseriate filaments, cells in median positions on the thallus aborted, or developed rhizoid tendencies long before the basal cells of the filament. The apical cells of others divided, initiating dichotomous branches (Figure 112). Some completely lacked any organised pattern of development. All these variations occurred in an estimated 70% of the population.

The other 30% exhibited a type of ontogeny similar to that described by Yamada and Kanda (1941) for Monostroma zostericola Tilden and Enteromorpha nana var minima Sjoest. The zooids gave rise to a monostromatic expanse of normal thallus cells. As some of these increased in size, rhizoids developed from their periphery, establishing

FIGURE 111 - A photograph showing clearly that vegetative and rhizoidal cells may be formed in the same filament. x600

FIGURE 112 - A dichotomously branched plant which originated from zooids of a Little Papanui population. x600

small replicas of the parent (Figure 113). As the larger discs increased in age, a swelling developed in the centre, giving rise to a multiseriate monostromatic tubular thallus (Figure 114). In cellular detail, the holdfast of the plants developing normally (Figure 115) agreed closely with that of the disc from which the other type developed. The difference between normal vegetative cells and the holdfast cells closely resembling them is shown in Figure 116.

The writer bases the following conclusions upon the evidence from the experiments described up to this point:

(1) When any South Island Enteromorpha population is grown in artificial culture, a range of embryological developmental pattern is likely to be found.

(2) As many of the variations are initiated by abnormal development at the time of the first divisions of the zooid or zygote, it appears likely that they are not culturally induced.

(3) However, only if a parallel range of variation occurs in the natural environment can these variations be considered as a normal feature of any population.

(4) There are distinct morphogenetic stimuli controlling the development of holdfast and blade as the same two types of cell occur in both.

   (a) Both rhizoids and normal blade cells may occur in the holdfast without any adverse effects on its form or function.

   (b) In Enteromorpha nana and some members of the Little Papanui population, a horizontal monostromatic disc

FIGURE 114 - A prostrate disc giving rise to a multiseriate upright filament. Raised from zooids of a Little Papanui population. x200

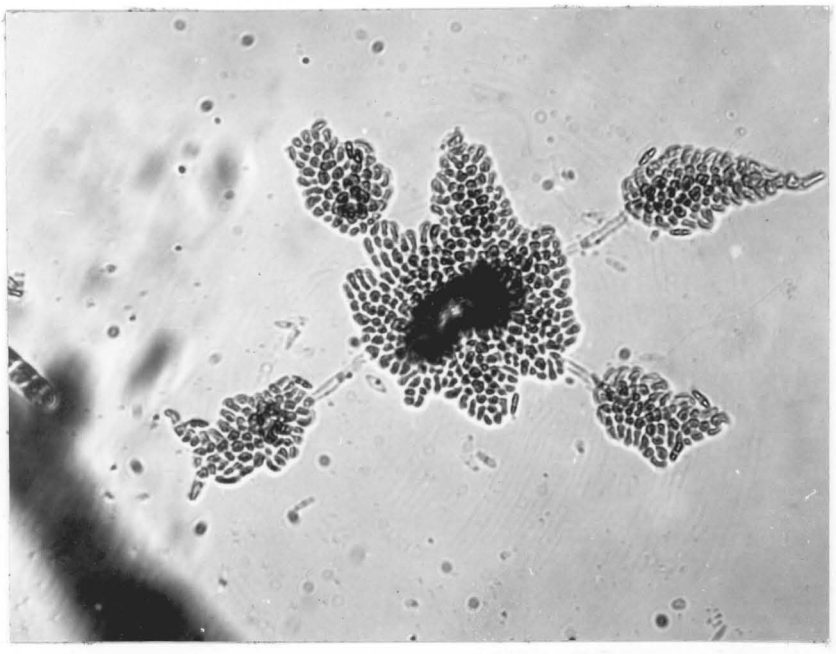


FIGURE 113 - Prostrate discs developed from zooids of a Little Papanui population. x200

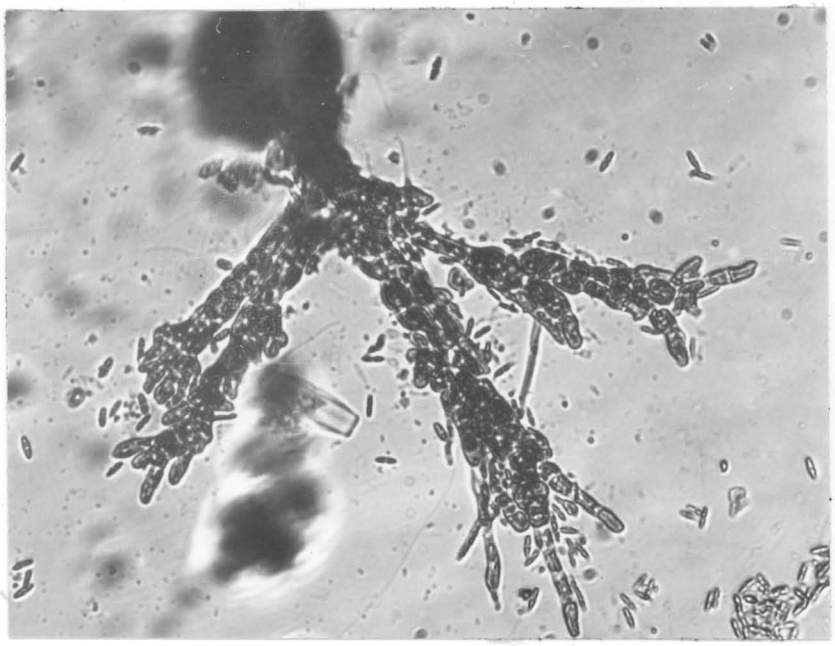


FIGURE 115 - The holdfast of a normally developing plant which originated from a zooid of the Little Papanui population. x200

FIGURE 116 - The holdfast of a young plant showing the small difference between some holdfast cells and normal thallus cells. x200

develops first. The upright filament developing later and in part from this disc is identical with it in cellular characters.

The validity of the postulation that any Enteromorpha population is likely to contain a natural range of embryological developmental pattern, rests upon the description of a comparable range from the natural environment. To this end, an Enteromorpha population averaging less than 1 mm in height was collected from the Motunau River in the winter of 1965. The results of this investigation support the original postulation.

Among the developing uniseriate filaments were found some in which the apical cell had aborted (Figure 117), developed into a rhizoid before those below showed any such tendency (Figure 118), or divided dichotomously (Figure 119) giving rise to dichotomously branched plants (Figure 120). Other sporelings at this stage of development had cells of median position in the filament developing into rhizoids before those below had done so (Figure 122).

More mature sporelings, of the multiseriate stage, included variants which had divided dichotomously (Figure 124) and those in which the apical cells had behaved in a variety of other ways. The cells sometimes aborted leaving one only functional in a formerly multiseriate thallus tip (Figure 125). In other plants, several apical cells gave rise to a short branch followed by a rhizoidal stipe subtending a secondary thallus (Figure 126). A similar process may also be initiated by a subapical cell (Figure 127). On other occasions the subapical cells may only give rise to a formless mass of cells (Figure 128).

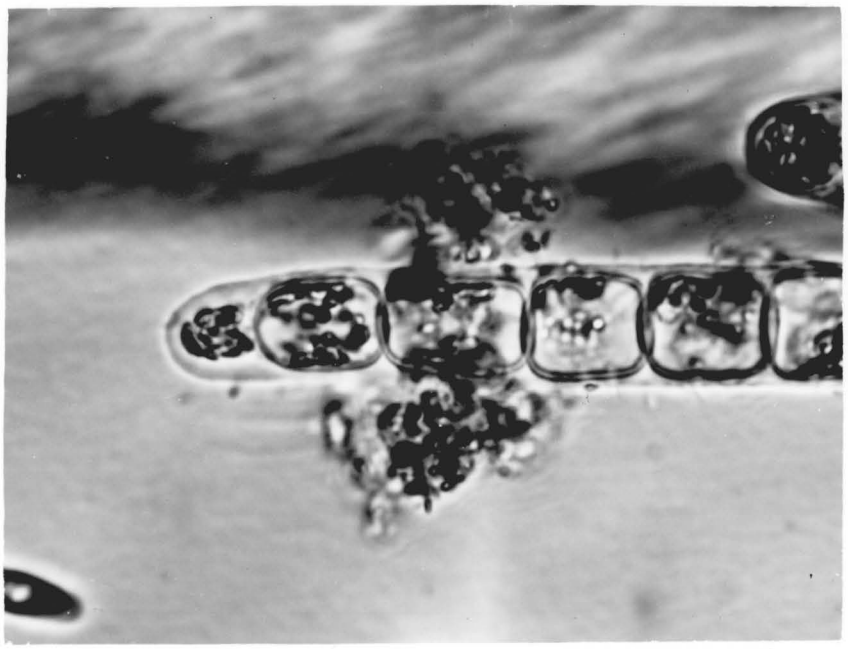


FIGURE 117 - A filament in which the apical cell has aborted.

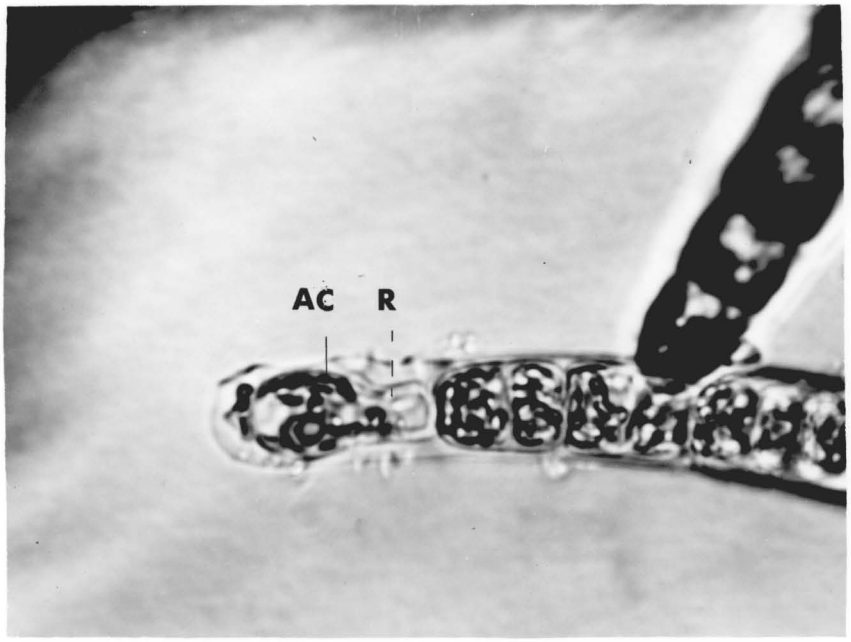


FIGURE 118 - A filament in which the apical cell (AC) has started to develop into a rhizoid (R).

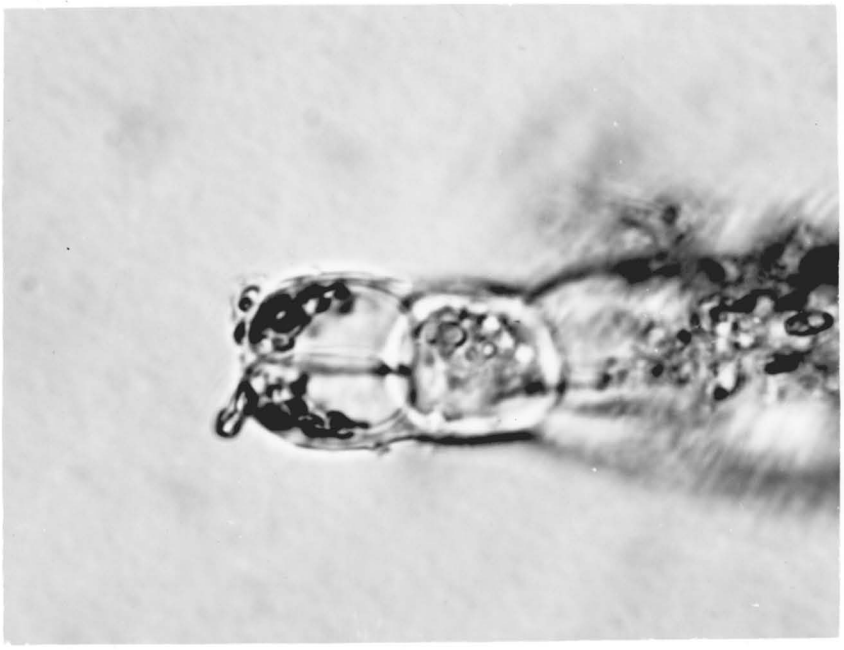


FIGURE 119 - A filament in which the apical cell has divided dichotomously.

FIGURE 120 - A dichotomously branched plant of the Motunau winter generation.

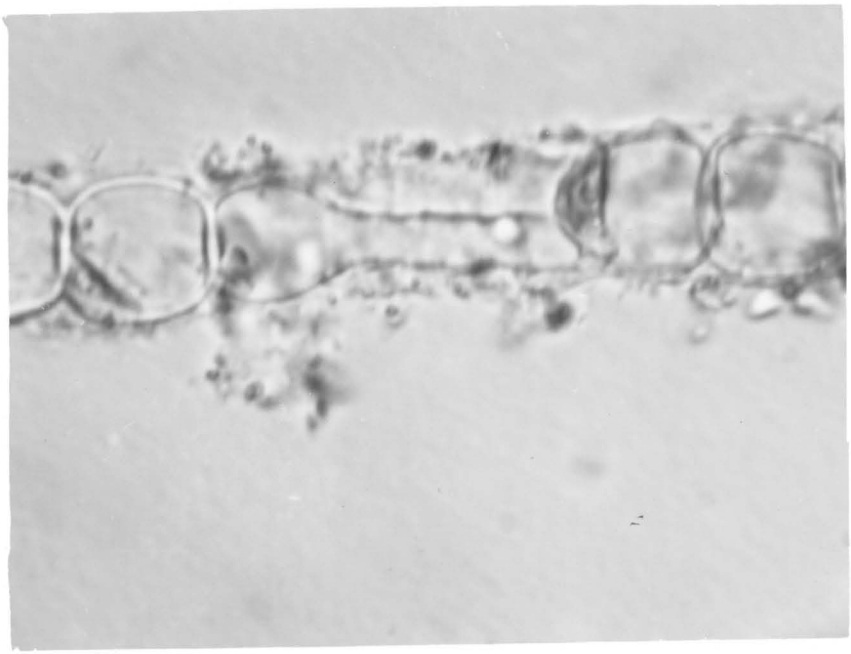


FIGURE 122 - The median region of a winter generation plant from Motunau with a cell developing into a rhizoid.

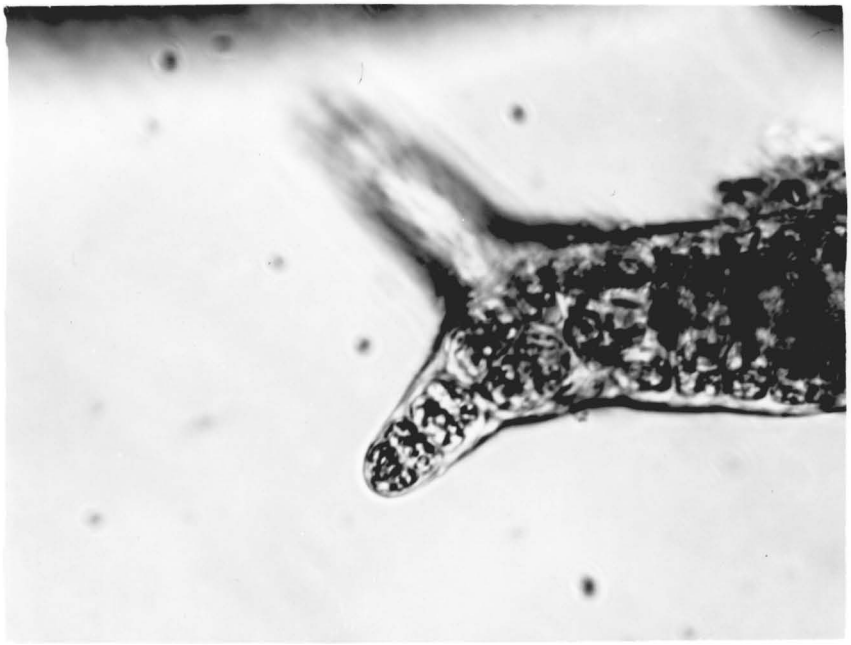


FIGURE 124 - A multiseriate plant which has divided dichotomously at the apex.

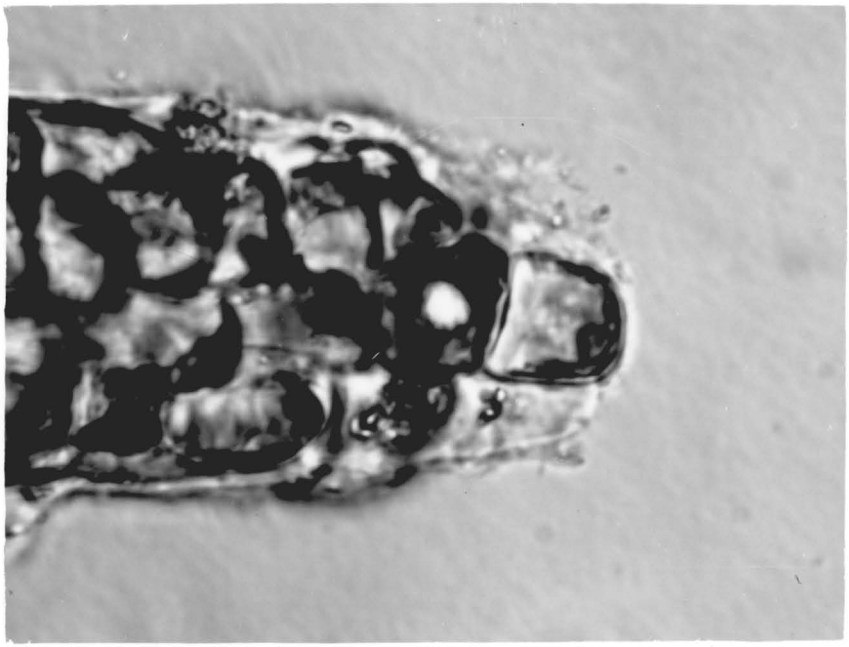


FIGURE 125 - A multiseriate plant in which all but one apical cell has aborted. x600

FIGURE 126 - Vegetative reproduction in a plant of the winter generation from Motunau. x200

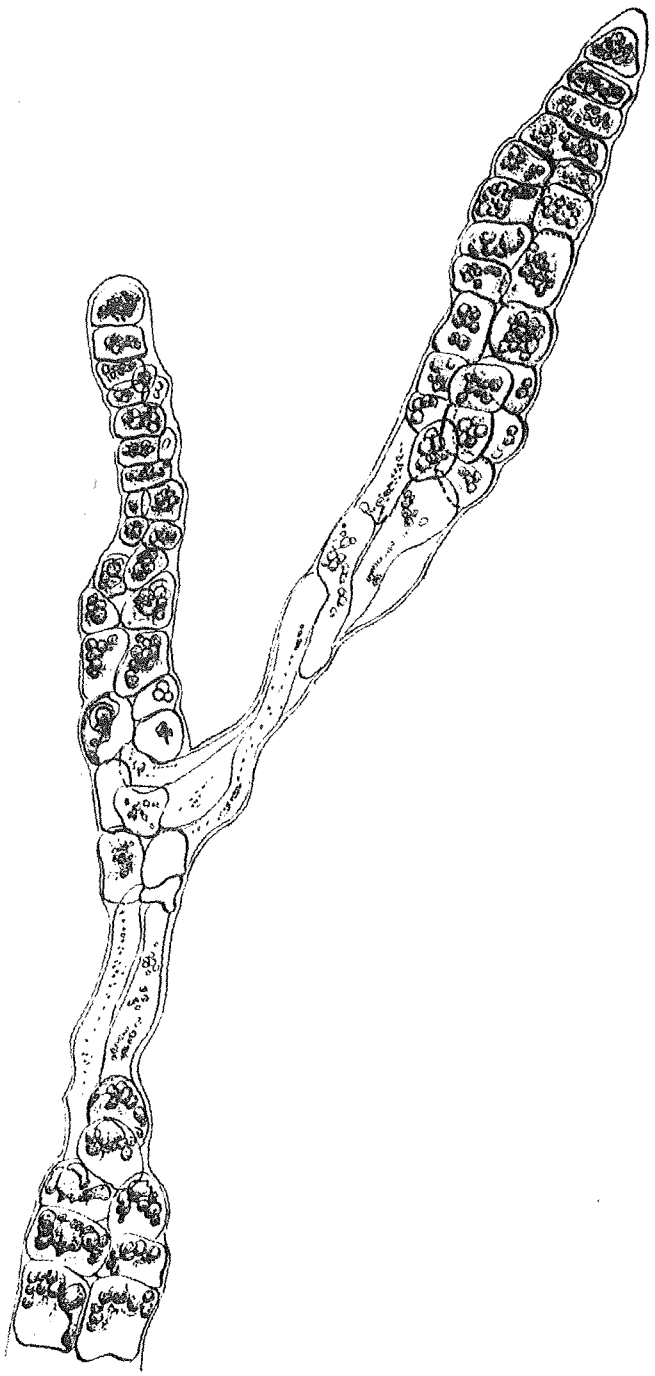


FIGURE 127

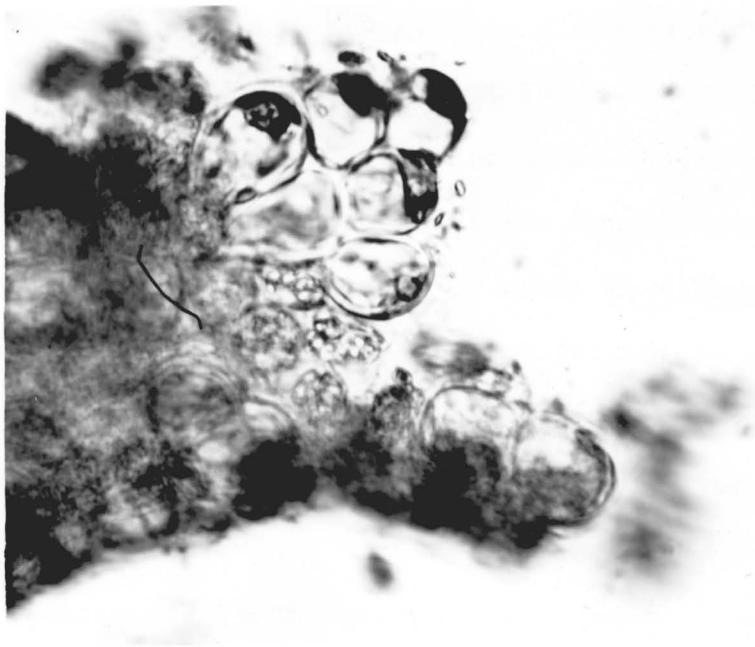


FIGURE 128 - A plant of the winter generation Motunau in which a subapical cell has given rise to a disorganised group of several cells. x600

The results of this investigation support the conclusion drawn from cultural experiments - that the balance between a normal blade cell and a rhizoid cell is very delicate. However, it is now possible to see the ecological significance of this. The secondary thalli shown in Figures 126 and 127 subtended by rhizoidal stipes are pending immediate release. Bearing in mind the totipotency of every Enteromorpha cell, and the similarity of in situ germination to the release of such secondary thalli, rhizoid formation is the effective stage in the successful release and reestablishment of these in the natural environment. It therefore provides a means of vegetative reproduction additional to those described in the previous section.

In concluding, there is little to be gained from summarising the ranges of embryological development in the various populations studied. The following points, however, are worthy or note:

(1) A comparable range of variation is found in populations growing in the natural environment and artificial culture. Pocock's (1959) failure to find a comparable range in Letterstedtia populations growing in the natural environment could be due to the size of the sample analysed. The writer examined several hundred plants to elucidate the range in the Motunau population.

(2) Although the writer believes that most of the variations discussed here are non genetic the ranges of variation cited here may include variants under strict genetic control (comparable to those of Føyn, 1961, 1962). To separate the two types would have involved experiments outside the scope and beyond the time limits of the present study.

(3) This report appears to be the first for the Genus Enteromorpha. It is apparent that there is a range of natural embryological variation in every population, a situation comparable with most taxonomic characters in other groups of plants.

THE LIFE HISTORY OF ENTEROMORPHA INTESTINALIS

Introduction.

Up to the present time only monomorphic life histories have been described for this genus. These include monomorphic diplohaplontic, monomorphic diplontic and monomorphic haplontic types. Scagel (1960) inclined to the view that a shrub-like gamete-producing plant 2 - 4 mm in diameter, previously identified as Collinsiella tuberculata is a normal part of the life history of Enteromorpha intestinalis on pebbly Canadian beaches. As the normal E. intestinalis and Collinsiella structures had not been grown through a complete life history in artificial culture, neither Scagel nor the present writer considers this as a confirmed dimorphic life history.

The object of this thesis was to confirm the existence of a dimorphic life history in an Enteromorpha intestinalis population growing in the Motunau River, North Canterbury. The observations upon which the decision to investigate the life history of this population was based were as follows.

(1) The summer zoospore and winter gamete producing generations were quite different in external appearance. Summer plants were characteristically large up to almost 6 feet long and $2\frac{1}{2}$ inches wide, unbranched in about 80% of the cases and generally convolute. In contrast to this, the winter generation was small, the largest plants usually being no more than 1 foot in length, branched in about 98% of the cases and non-convolute.

The range of gross morphological variation in the summer generation is shown in Figure 129 and for the winter generation in Figure 130.

FIGURE 129 - Plants illustrating some of the range of form of the Summer Generation at Motunau.

FIGURE 130 - The range of gross morphology of the winter generation at Motunau.

A common form of winter generation plant is shown in Figure 131.

However, two populations so different in outward appearance could have been different species which reached developmental maxima in different seasons of the year. In view of the second observation this was considered unlikely.

(2) The summer generation grew between September and March approximately, the winter generation between April and August. The two were separated by a period of about two weeks during which time there were no plants longer than 1 - 2 cm in the river. Although the length of the period varied there was always a complete break in time between the occurrence of the two populations. A regular break between the occurrence of two separate species would be most unusual. The third observation linked these two morphologically and seasonally distinct populations together in the same species.

(3) Most summer plants disappeared early in the autumn. Some of the sparsely branched summer plants remained a little longer, and during this period they developed additional fine branches characteristic of the winter generation (Figure 132). On one occasion a summer plant was found during this period which had proliferated a number of unbranched summer plants from the stipe and one small plant more characteristic of the winter generation (Figure 133). Microscopic examination showed that this plant had definately developed from a stipe cell. In addition, if the branched plant had been established independently, it would have developed a rhizoidal holdfast with which to attach itself to the other plant. There was no evidence of this (Figure 134).

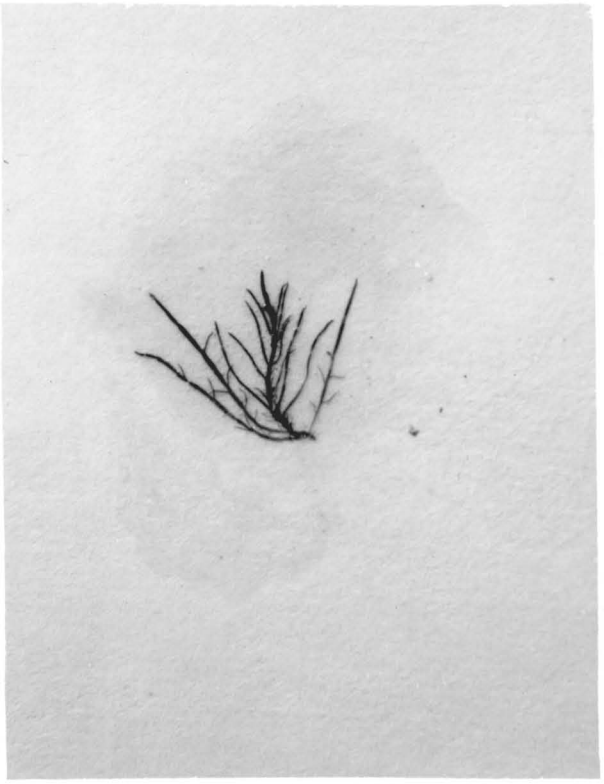


FIGURE 131 - A common form of winter generation plant from Motunau. x2 natural size

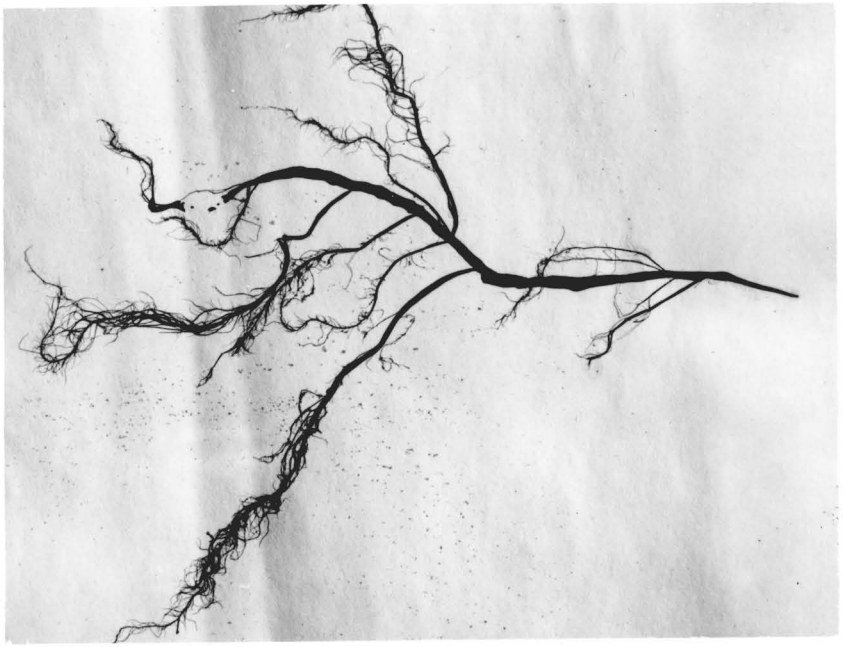


FIGURE 132 - A sparsely branched summer plant of Enteromorpha from Motunau with fine branches characteristic of the winter generation.

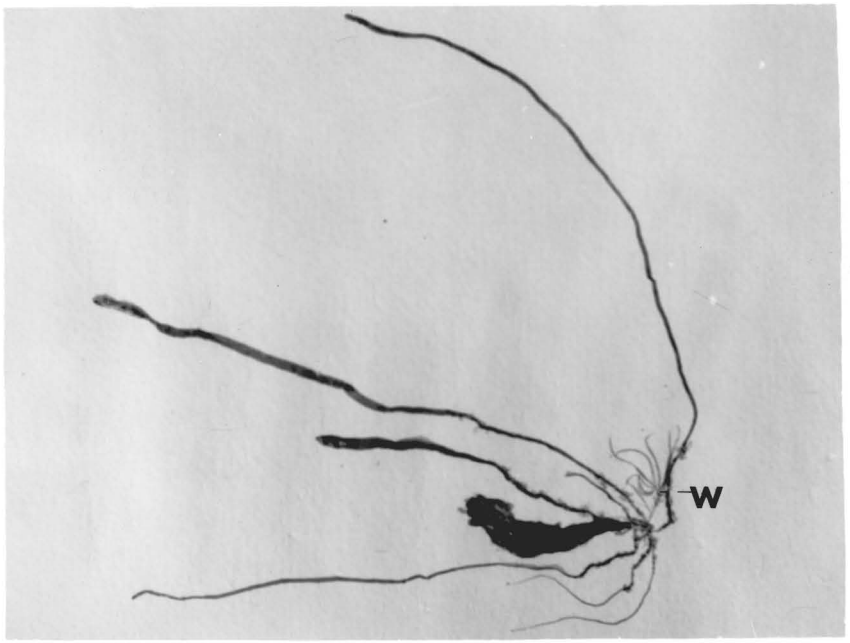


FIGURE 133 - A summer plant of Enteromorpha from Motunau with a winter plant (W) developing from the stipe.

FIGURE 134 - A detailed photograph of the plant in Figure 133 showing the attachment of the winter plant to the stipe of the summer plant.

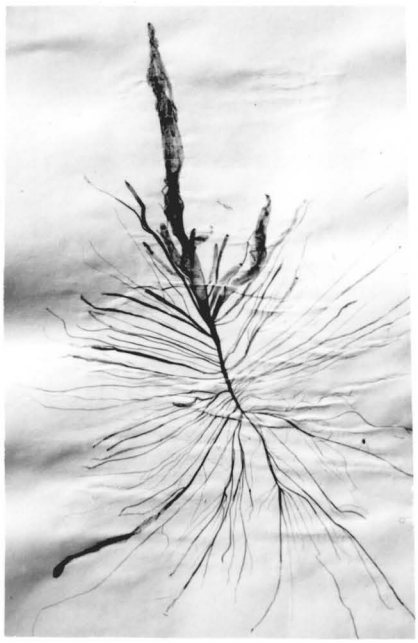


FIGURE 135

FIGURE 136

Two summer generation plants from Motunau showing long fine branches (135) and more numerous shorter ones (136) reminiscent of the winter generation.

The fine branching characteristic of the winter generation appeared to be a facultative character whose development was greatly influenced by the natural environment.

The final observation, which appeared to confirm conclusively the summer and winter generations as members of the same species was this: A small number of summer plants possessed additional features reminiscent of the winter generation. They had a few branches similar to those of the summer generation and a large number of fine ones similar to those of the winter generation (Figures 135 and 136). They exhibited therefore features transitional between summer and winter plants.

Several of these morphologically intermediate forms liberated both gametes and zoospores. In view of this sprinkling of intermediates amongst the summer plants, it was postulated that the summer - winter dimorphism was at least to some extent genetically controlled. Thus the summer generation had an intrinsic capacity for developing into large unbranched or occasionally sparsely branched plants, the winter generation one favouring the development of small profusely branched plants. Later laboratory experiments provided evidence that this capacity was realised only to the extent environmental factors permitted.

Early in July 1965 a number of winter generation plants from Motunau including branched and unbranched plants of various sizes were placed in a vessel of autoclaved sea water at the laboratory window. A few days later gametes were released and the planozygotes settled at the water surface. By the end of the month these germlings had formed a visible growth and the parent plants had started to decay. A detailed description of the method of culture has already been given on page

of this thesis.

Objectives.

The object of this experiment was two-fold:

(1) It was hoped that several generations of the same species could be raised in the one vessel, in conditions as close to those of the natural environment as possible. For this reason the parent plants of each generation were left to decay, and only negligible amounts of autoclaved sea water had to be added to compensate for evaporation losses.

The morphological features characteristic of summer and winter generations appeared to be under a certain amount of genetic control. Although the vessel in the laboratory was only a crude replica of the natural environment, it was hoped that a seasonal fluctuation of certain environmental conditions would occur, sufficient to encourage the development of seasonal morphological differences approaching those of the natural environment.

(2) The importance of embryology in certain genera of the Ulvales, including Enteromorpha has already been discussed. It was hoped that this experiment would provide a means whereby the embryology of successive generations of the one species could be compared.

The Life History of Enteromorpha Intestinalis (L) Grev. from Motunau, in Artificial Culture.

The morphology of the generation developed from planozygotes resembled closely that of the winter generation in the natural environment, save for the slightly greater proportion of unbranched plants. As explained in a previous section, no satisfactory chromosome

stains were available therefore the postulated nuclear status of this and the subsequent generations could not be confirmed.

The plants of this first cultural population termed C.P.1 began to lose colour about September 1965. The water level of the vessel was raised in anticipation of zooid formation. However, this did not occur and all plants save for the holdfasts and an occasional section of thallus completely lost their colour and rotted. By 31 December, however, small dark green dots had appeared in the old holdfasts and occasionally in pieces of blade, from which new plants developed by in situ germination. Figure 137 shows the unicellular stage of this sequence, Figure 138 a short uniseriate filament and its subtending cell, and Figure 139 a holdfast of C.P.1 with a number of C.P.2 plants developed by in situ germination. It was interesting to note that the plants on the holdfasts developed faster than those from intercalary regions of the blade.

The range of non genetically determined variation in C.P.2 was similar to that already described for a cultural population from Motunau (page 223 of this thesis). Growth of the population overall was rapid, as all plants were large enough for description of their gross morphology by March 1966. There was an overwhelming dominance of unbranched and sparsely branched plants comparable to those of the summer generation in the natural environment. Although not all were examined outside the vessel for fear of endangering zooid formation and establishment of the next population, no finely branched plants similar to C.P.1 and the winter generation in the natural environment could be distinguished.

The plants of C.P.2 in Figure 140 were removed from the vessel on June 31, 1966. The large sparsely branched specimen was 20 cm long and

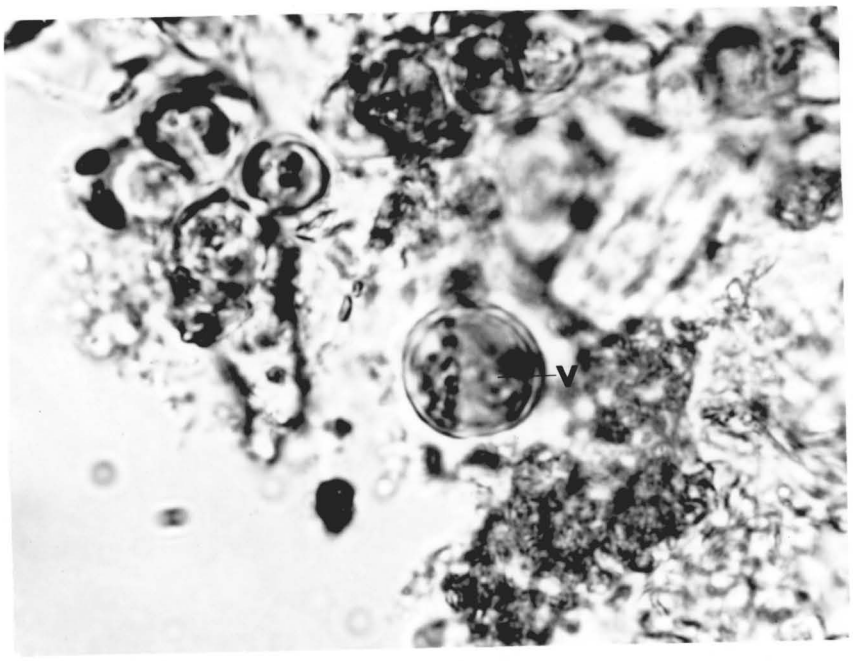


FIGURE 137 - A single 'vegetative cell' (V) of cultural population one, specialised for in situ germination. x600

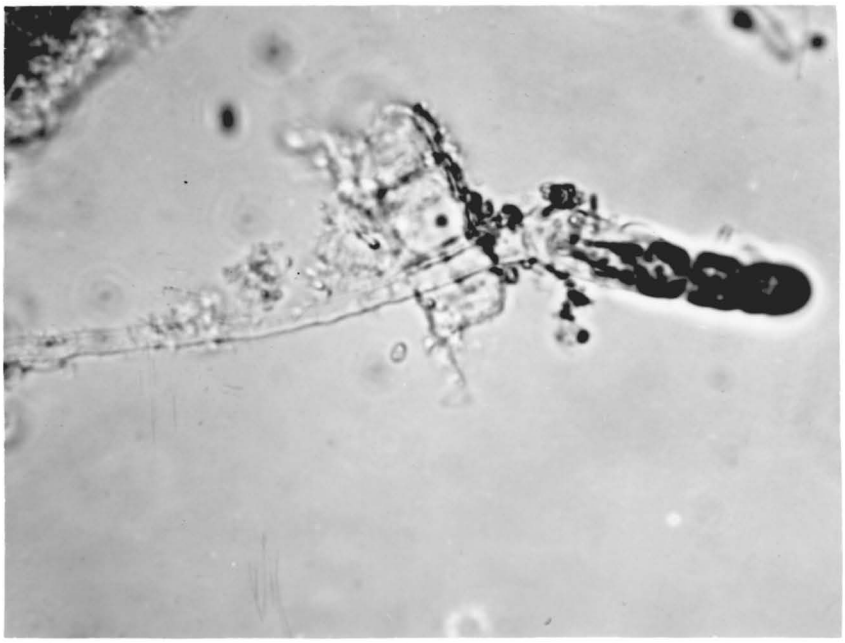


FIGURE 138 - A young plant of cultural population two. x400

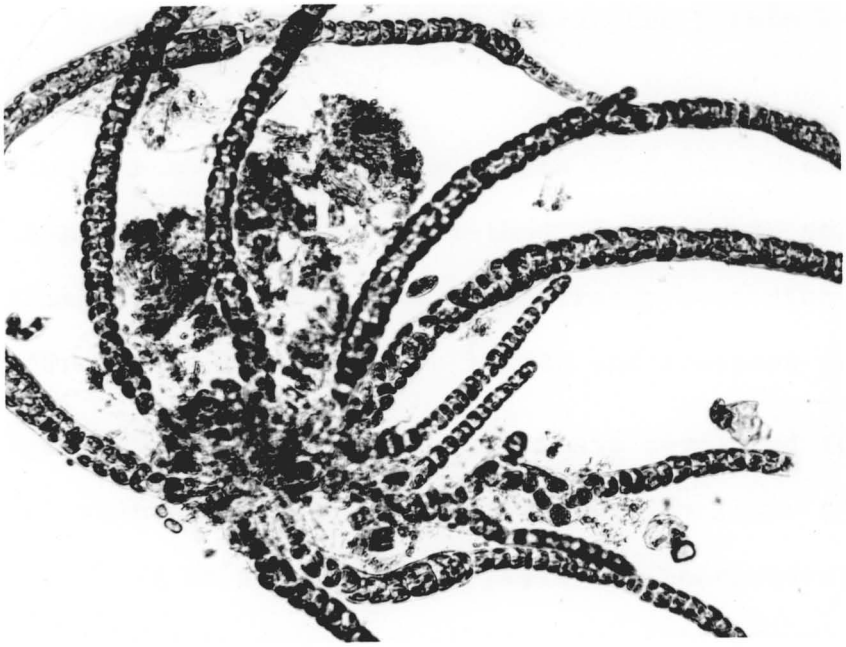


FIGURE 139 - A number of cultural generation two plants originating from the decaying holdfast of a plant of cultural population one. x400

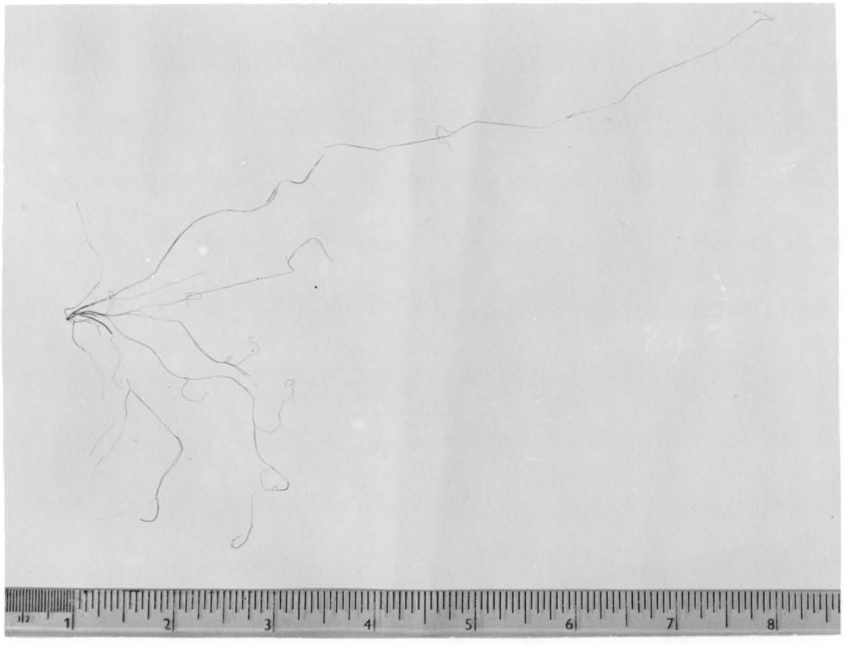


FIGURE 140 - Plants of cultural population two at reproductive maturity.

1 mm wide, dark green during early growth, but undergoing a basipetal lightening of colour later. Several smaller unbranched plants are also visible in this figure.

During the period March - June 1966 the plants of C.P.2 released quadriflagellate zoospores. The peculiar method of discharge and observations pertaining to zooid structure have already been discussed under the heading "Processes leading up to zygote and zoospore formation".

During investigation of the embryology of plants developed from C.P.2 zoospores particular attention was given to the prostrate system. This was due to the results of an experiment performed independently of this investigation, designed to determine whether the embryology of plants which never settled on a solid surface differed from those which did. This experiment will be described briefly in order to present a complete picture.

A comparison of the embryology of an Enteromorpha population growing unattached at the surface of the water, with an attached population. Branched plants of summer generation one were collected from point C Motunau early in December 1965. In order to establish two classes of zoospore, (1) those which settled and (2) others which remained at the surface of the water, the fertile plants were placed in a large petrie dish of filtered sea water, covered with several thicknesses of glass and left undisturbed, for a period of several weeks. After this period, a film containing a large number of bright green dots covered the surface of the water, and areas on the bottom of the petrie dish. These developed into sporelings, which were left undisturbed until June 1966, when the following observations were made.

The zoospores which attached themselves to the bottom of the petrie dish developed into plants with small holdfasts composed largely of cells similar to normal blade cells. Most of these which remained unattached at the surface developed only a prostrate system, (Figure 141) composed largely of cells closely resembling those of the upright system (Figure 142). From some of these amorphous masses of cells grew upright filaments (Figure 143) but these were rarely as well developed as those of attached plants.

The prostrate system is thus a plastic feature of Enteromorpha sporelings; when plants remain unattached it becomes very large. It is likely that the polarity of plants developing at the water surface is continually altered. The daily heating and cooling of the water would be expected to create convection currents which probably keep moving the smaller plants about. As a result, the polarity is not the same for two successive divisions. With an increase in size the amount of possible movement would decrease until a constant polarity is established, when "upright" filaments could develop. The more important points raised by this plasticity may be considered.

(1) The possibility that zoospores which settle in a variety of places in the natural environment may have different patterns of development and,

(2) that this might also occur in species other than Enteromorpha intestinalis.

The second possibility could not be further investigated during the present study. However, the writer inclines to the view that the

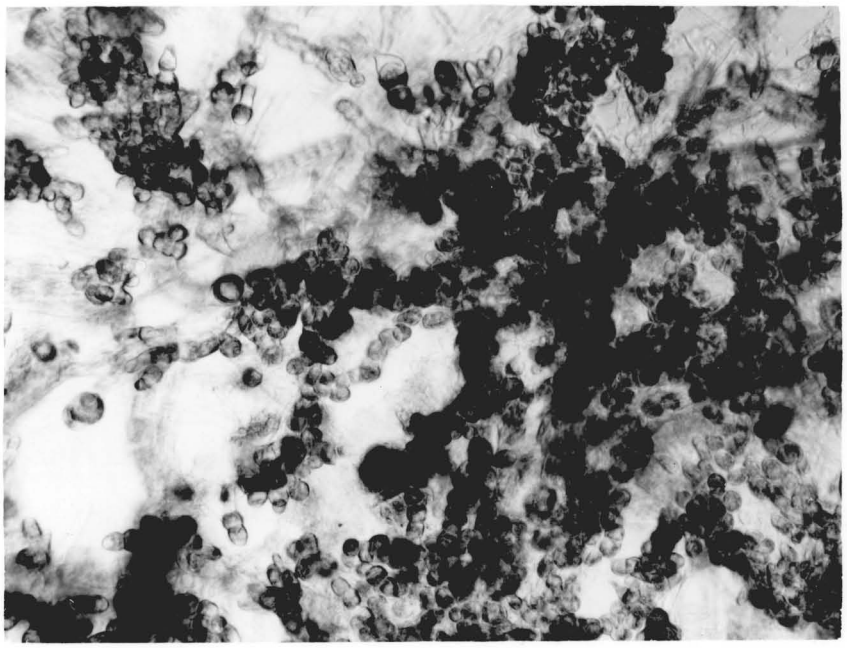
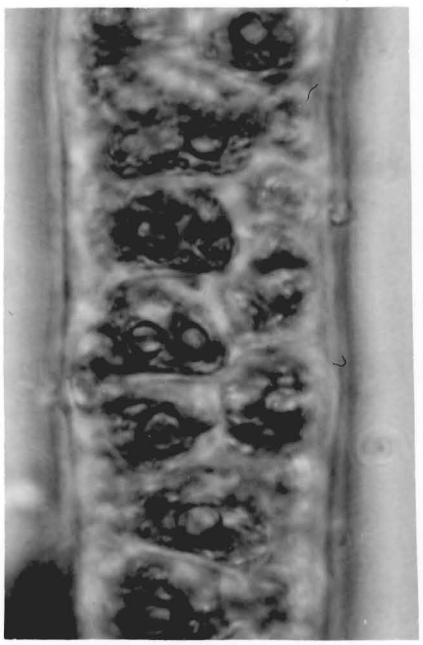


FIGURE 141 - The prostrate system developed from a zoospore of Enteromorpha intestinalis which remained at the water surface. x400



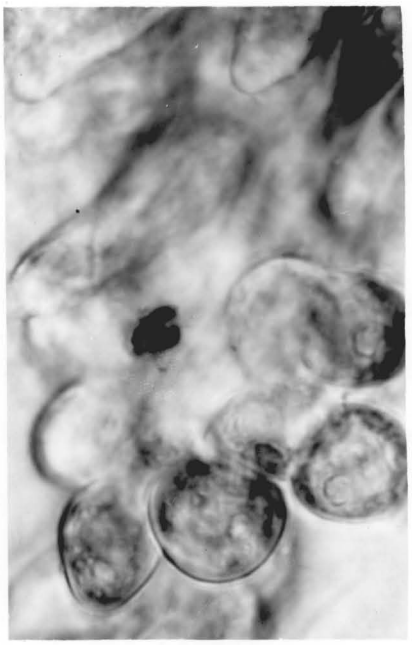


FIGURE 142 A FIGURE 142 B

The cells of the prostrate system (142 B) in Figure 141, closely resembled those of the upright system (142 A). x600

validity of any taxonomic unit established upon this criterion is seriously in doubt unless it was previously established that the mode of holdfast development was not plastic but genetically stable.

The results of the following investigation demonstrate that the development of the holdfast in E. intestinalis grown in a variety of artificial environments is sufficiently plastic to permit widely differing patterns.

The Embryology of Enteromorpha intestinalis (L) Grev. grown in artificial culture. In order to compare the range of embryological variation of this population with that of a similar population, discussed under "Embryological Variation in the Genus Enteromorpha" zoospores were collected from C.P.2. They were grown between a slide and No. 1 cover slip in a Petrie dish containing filtered sea water, and illuminated by 2 80 watt 5' fluorescent tubes 6" above the bench. The reasons why the influence of the coverslip on the plants was considered negligible has already been explained in the section "Variations in Embryology". Briefly they were:- (1) the range of variation of the cultured plants corresponded closely with that of plants growing in the natural environment; (2) the film of water between the slide and coverslip was sufficient to keep the latter from resting on the plants. The coverslip could be shifted laterally without moving the plants. The following observations were made on June 30 1966, 9 weeks after the cultures were established.

Many sporelings were at various stages of the normal developmental sequence. In some the first two cells differentiated into a vegetative and rhizoid initial (Figure 144) while in others the first rhizoid

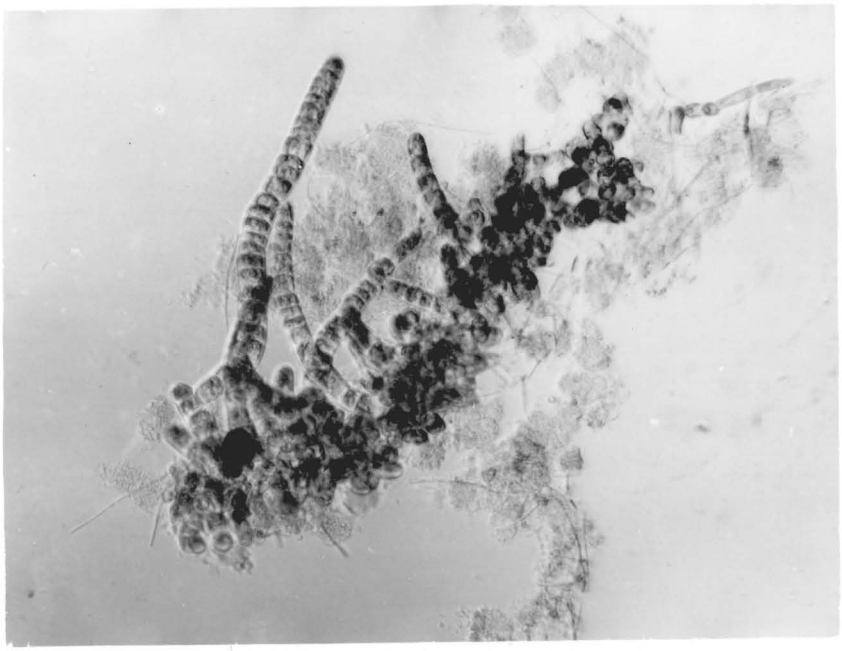


FIGURE 143 - Upright filaments developed from an extensive prostrate system growing at the water surface. x400

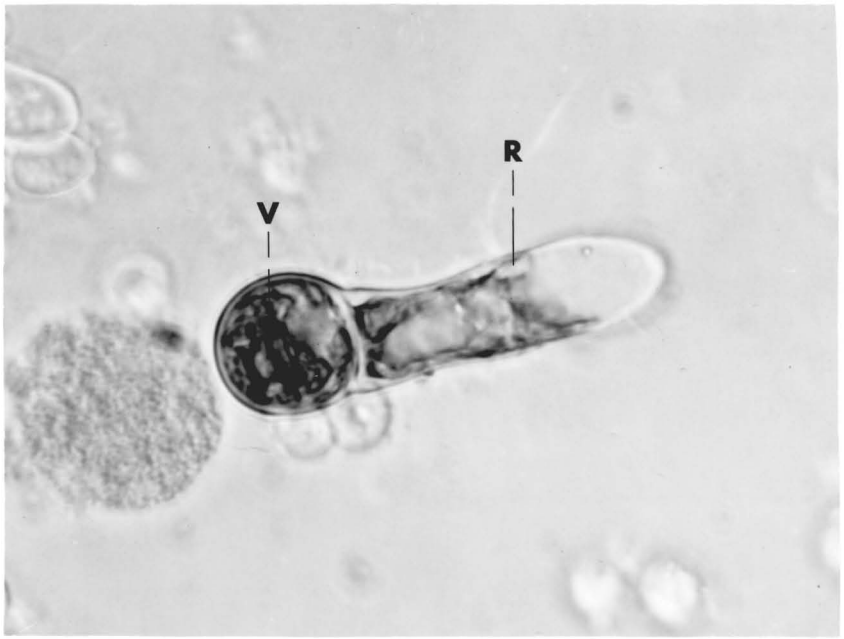


FIGURE 144 - An Enteromorpha intestinalis plant sharply differentiated into rhizoid (R) and vegetative (V) cell initials. x600

formed after the differentiation of several vegetative cells (Figure 145). In a small number of plants development of the holdfast occurred before the formation of the upright filament (Figure 146). The rhizoids developed branches either by division of the terminal cell at the holdfast end of the filament (Figure 147), by longitudinal divisions in several subterminal cells (Figure 146), or by similar divisions restricted to the cells at the junction of the holdfast and blade (Figure 148).

Other variations included plants which developed upright multiseriate filaments attached by only 2 - 3 rhizoid cells, others with an irregular distribution of variably orientated longitudinal divisions in a uniseriate filament, and plants which lacked any organised pattern of development (Figure 149).

When these cultures were originally established, one zoospore-producing filament of C.P.2 liberated zooids which possessed a very strong tendency to clump (Figure 150). As this was the only occasion on which such behaviour was observed, the zooids were maintained as a separate culture. After 9 weeks growth the clumps had developed into colonies of remarkably regular appearance (Figure 151). Whilst several zooids outside these colonies had commenced normal development, those belonging to one appeared to develop collectively. Small upwellings of tissue formed in the centre of most colonies, giving them an appearance similar to early stages of development of Enteromorpha nana var minima Sjoest (Yamada and Kanda, 1941).

The range of variation of plants grown between slide and coverslip is comparable with that previously recorded for this species. It is not comparable with that of plants growing at various levels on the side

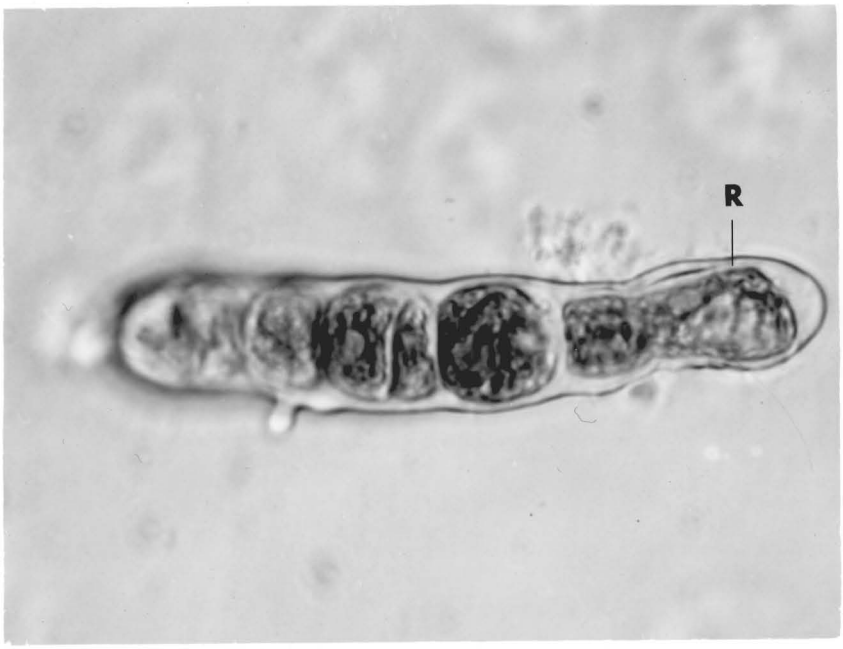


FIGURE 145 - An Enteromorpha intestinalis plant in which the rhizoid initial (R) formed after the development of several vegetative cells. x400

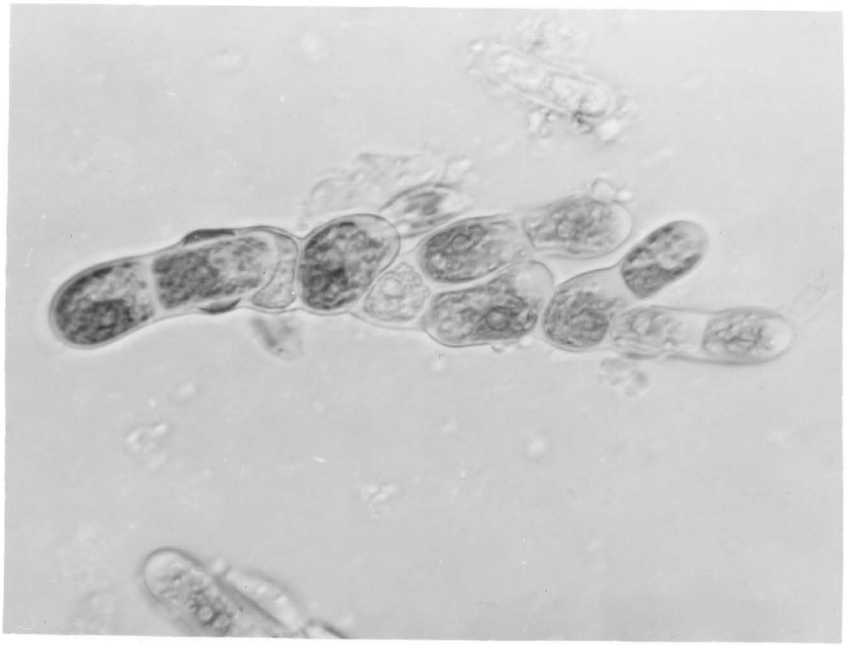


FIGURE 146 - A plant of Enteromorpha intestinalis composed entirely of holdfast. x400

FIGURE 148 - An Enteromorpha intestinalis plant in which holdfast branches are developing by longitudinal divisions of a cell at the junction of holdfast and blade. x400

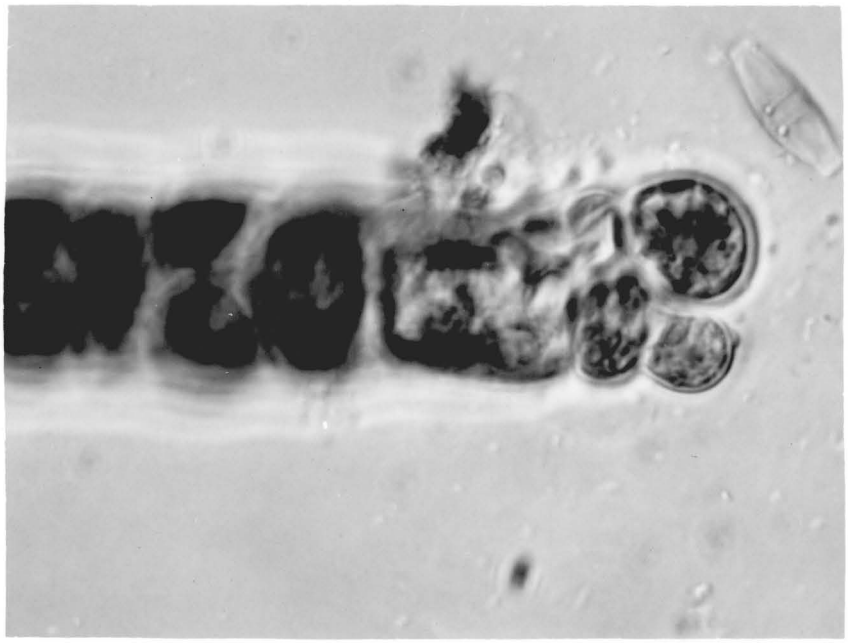


FIGURE 147 - An Enteromorpha plant in which holdfast branches are developing by dichotomous division of the rhizoid initial. x400

FIGURE 149 - An Enteromorpha plant which lacks any organised growth pattern. x400

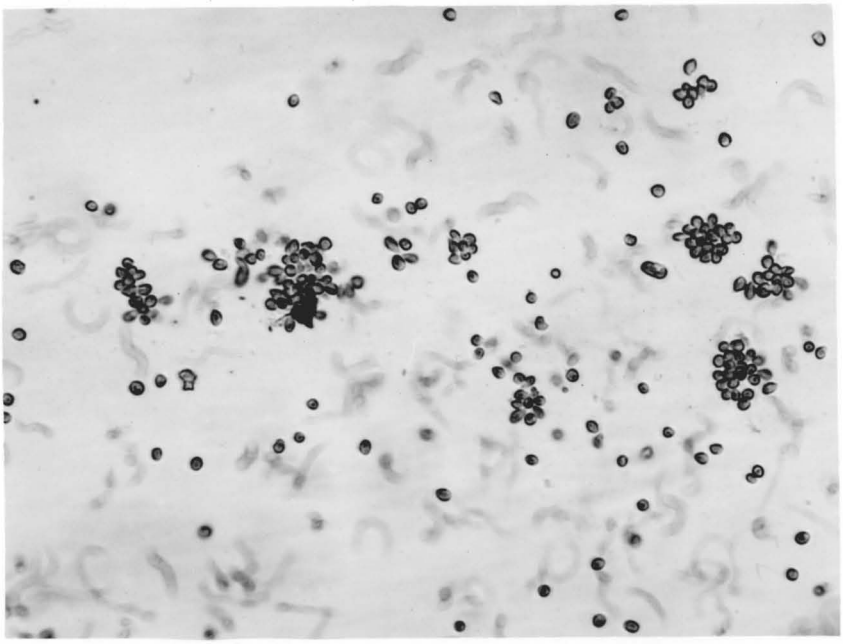


FIGURE 150 - Zoospores released from cultural population two during clumping. x200

of the main culture vessel, which exhibited a great range of holdfast development.

Embryological variations of plants growing on the side of the main culture vessel near the surface of the water. In order to compare the range of embryology of plants grown under a variety of cultural conditions, a number of sporelings which developed from C.P.2 zoospores were removed from the main culture vessel near the water surface.

The holdfasts of these plants were considerably more developed than those of plants grown between a slide and coverslip. Some possessed shrub-like holdfasts subtending long upright filaments (Figure 152) while other plants had produced more upright filaments and less holdfast (Figure 153). In addition some zoospores gave rise directly to structures which possessed no organised pattern of development, (Figure 154), while in other cases these were produced indirectly from a moderately developed prostrate system (Figure 155).

Compared with the plants growing several centimeters from the surface on the same side of the vessel, these plants had well developed upright systems.

Embryological variations of plants growing on the side of the culture vessel several centimeters below the water surface. In this position most sporelings had concentrated upon the development of a prostrate system. Many produced only a rounded multistromatic shrub-like structure about $\frac{1}{2}$ mm in diameter (Figure 156) thought to be a 'species' of 'Collinsiella',* a few of which subtended short uniseriate

---

* If these plants were the same as those identified as Collinsiella, this would not be a valid taxonomic unit.

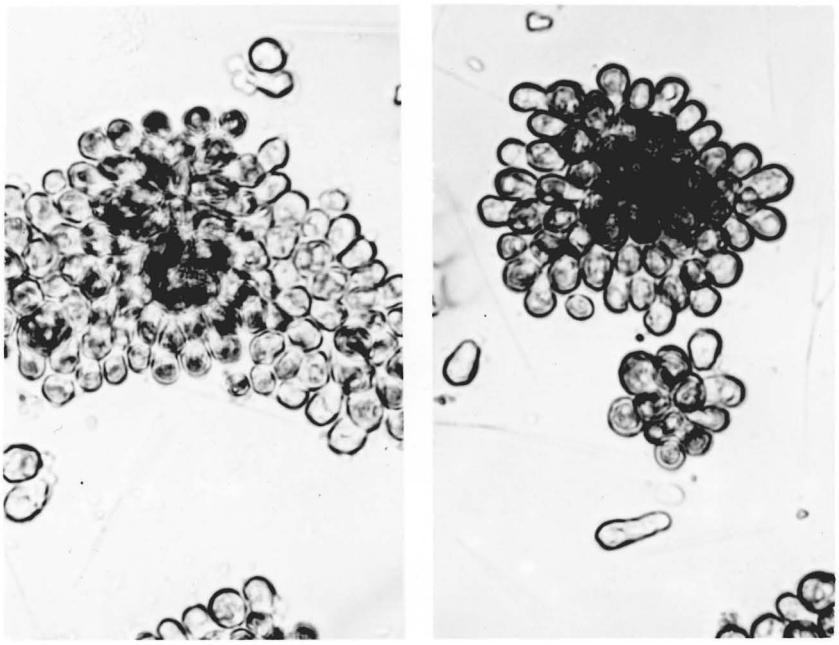


FIGURE 151 - Two clumps of zoospores after nine weeks' growth. x400

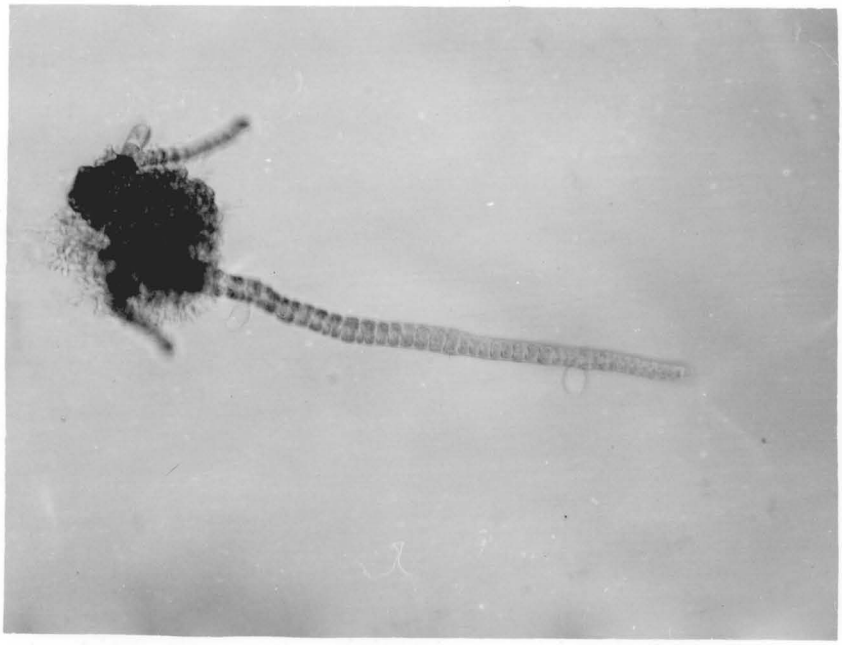


FIGURE 152 - An Enteromorpha plant with a shrub-like holdfast and a long upright filament. x200

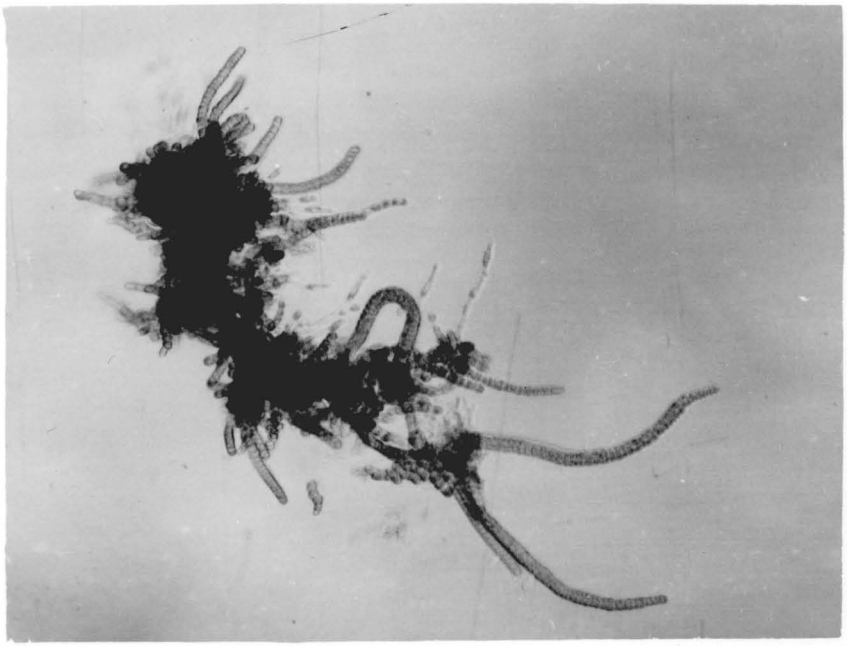


FIGURE 153 - An Enteromorpha plant(s) growing close to the water surface with a number of developing upright filaments. x200

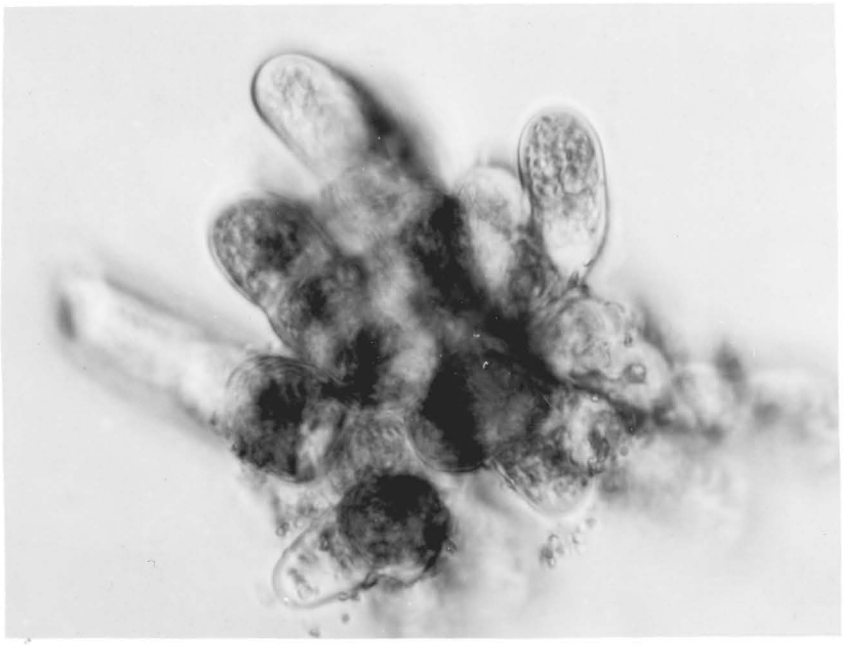


FIGURE 154 - An Enteromorpha 'plant' growing close to the water surface with no organised pattern of growth. x600

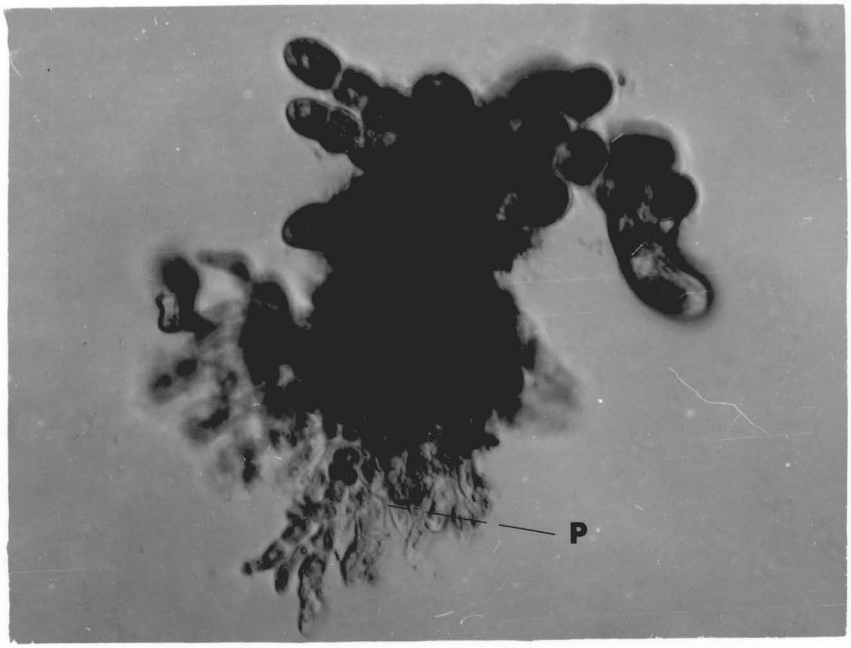


FIGURE 155 - An Enteromorpha 'plant' lacking any organised pattern of growth developed from a prostrate system (P). x400

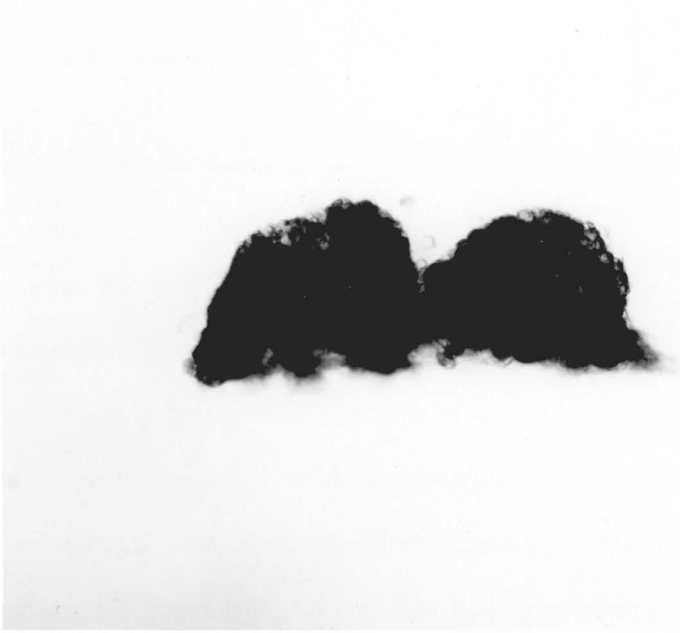


FIGURE 156 - Collinsiella-like plants developed from zoospores of Enteromorpha intestinalis. x100

filaments (Figure 157). In others the central region of the disc gave rise to a monostromatic upright thallus open at the apex, similar to that formed in Monostroma (Figure 158). The most interesting forms of shrub-like growth were those in which the peripheral monostromatic layer of cells developed into zoosporangia (Figure 159). Upon removal from the substratum they liberated motile quadri-flagellate zoospores similar in appearance to those produced by the summer generation at Motunau.

Other zoospores from C.P.2 developing several cm from the surface did not develop into several layered discs. Instead they remained monostromatic (Figure 160) and only occasionally gave rise to a few short upright uniseriate filaments (Figure 161).

The subject matter of the following discussion falls into two broad categories.

(1) Those aspects pertaining to the life history of the species,

and (2) taxonomic implications.

Discussion of the Life history of Enteromorpha intestinalis (L) Grev. at Motunau.

Unfortunately the writer was not able to stain the chromosomes of any Enteromorpha species. Conclusions regarding the nuclear status of each generation were based upon the assumptions that (1) the gamete-producing generation is haploid, (2) copulation of mitotically produced gametes leads to the establishment of the diploid generation, and (3) the haploid generation is reestablished by the germinating of zoospores produced meiotically by the diploid generation. While it may seem unusual to draw conclusions about the nuclear sequence of a life history

FIGURE 157 - A Collinsiella-like plant giving rise to a uniseriate filament. x100

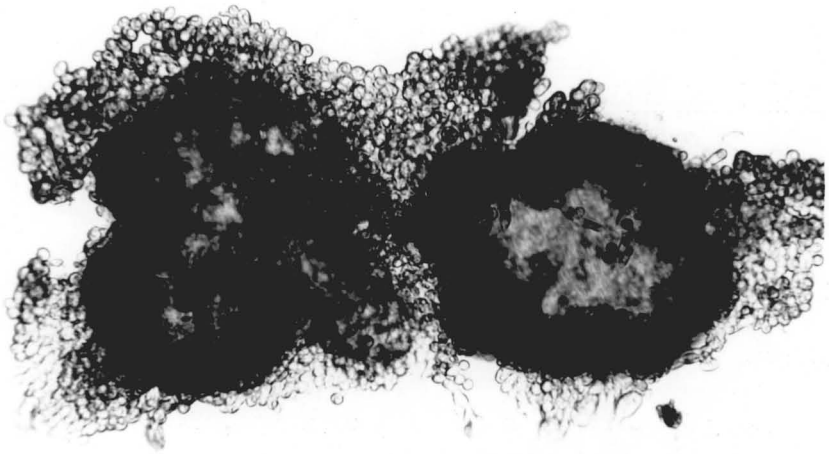


FIGURE 158 - Two plants raised from E. intestinalis zoospores with an embryological pattern similar to early developmental stages of Monostroma. x200

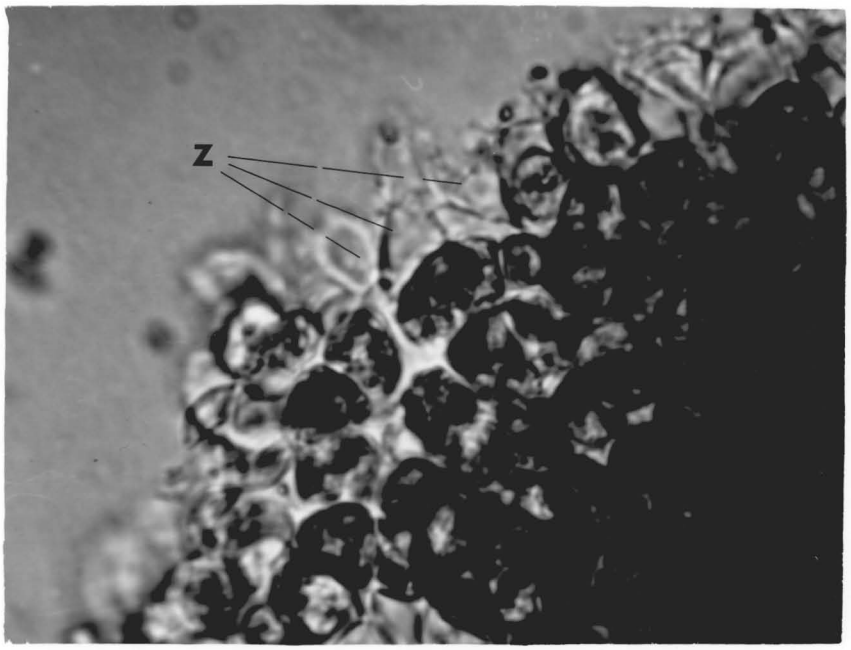


FIGURE 159 - A monostromatic layer of cells in which the peripheral members have been transformed into zoosporangia (Z). x600

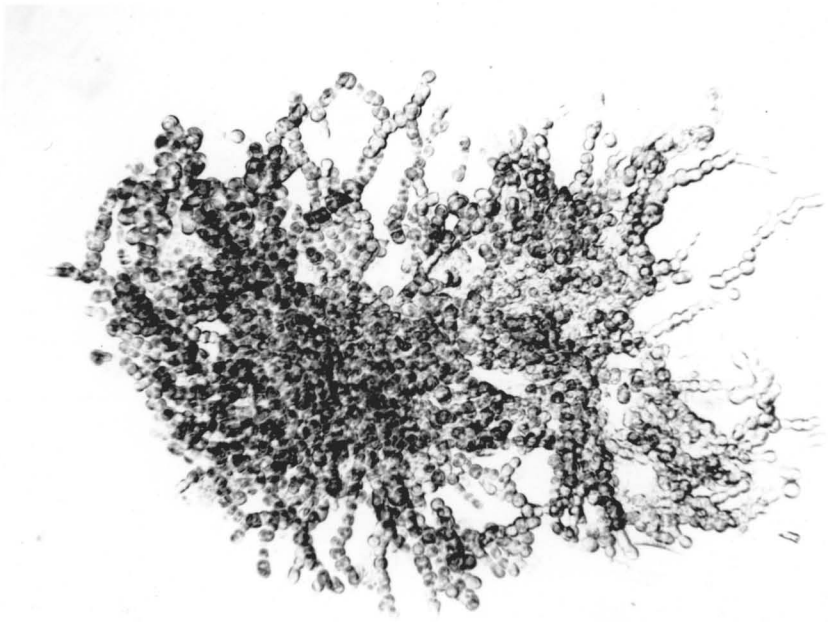


FIGURE 160 - A monostromatic layer of vegetative cells developed from E. intestinalis zoospores. x400

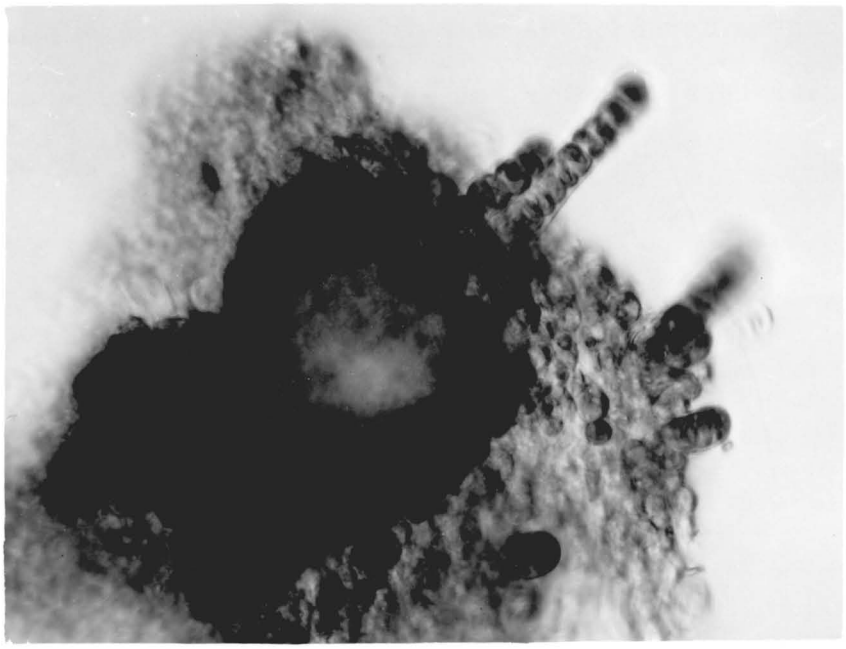


FIGURE 161 - A monostromatic layer of cells developed from E. intestinalis zoospores, giving rise to upright filaments. x400

without chromosome counts, the situation is not without precedent. Islam (1963) revised the Genus Stigeoclonium in the absence of nuclear information by making similar assumptions; and a great many other phycologists were unable to solve the same problem.

The life history of most Enteromorpha plants in the natural environment consists of a sequence of diploid zoospore-producing and haploid gamete producing generations with meiosis at zoospore formation. An accessory reproductive cycle occurs during the early summer, when mitospores give rise to additional diploid plants. In culture C.P.1 was believed to be diploid because it originated from copulation of gametes and C.P.2 also diploid as it developed from C.P.1 by in situ germination and gave rise to C.P.3 (haploid) by zoospores. If this is correct, then in culture an extra diploid generation was interealated without zooid formation between the normal two generations.

The main object of this thesis was to confirm the existence of a dimorphic diphasic life history in Enteromorpha intestinalis growing at Motunau. In the natural environment the winter generation consists largely of small finely branched gamete-producing plants, the summer generation mainly of large unbranched zoospore-producing plants. If the mesic nature of the cultural conditions is taken into account the difference in gross morphology between cultural winter generation C.P.1 and cultural summer generation C.P.2 is comparable with the difference between the morphology of the winter and summer generations at Motunau. If the assumptions about the nuclear status of these cultural generations are correct, then the diploid C.P.1 which developed during the winter, exhibited the morphology of the haploid winter generation in the natural

environment. The development of the distinctive morphological phases is therefore considered facultative, being controlled largely by the environment.

In both the laboratory and natural environment, the summer and winter plants are each regarded as a distinct morhpological phase. The Collinsiella stage of C.P.3 recorded only in culture but probably comparable with that observed by Scagel (1960) on a pebbly beach is regarded as the third distinct morphological phase. The present writer has not proved its absence at Motunau, but a thorough search for early stages may yet reveal it there.

It is universally agreed that observations made under cultural conditions are not always applicable to the natural environment. However, in this matter the writer follows Gayral (1960) who based some conclusions about the situation in nature upon cultural experiments, in the same way that conclusions about the normal may be drawn from pathology and teratology. The Collinsiella - like plants were considered as the third distinct-morphological phase on the following grounds.

(1) No upright filaments were developed by many of these after several months' growth. Had they been an intermediate stage in the development of normal plants, some uniseriate filaments would have developed after such a time.

(2) The Collinsiella plants formed about 80% of C.P.3.

This study therefore permits the following conclusions about the life history of the E. intestinalis population to be drawn.

(1) The population has a trimorphic diphasic life history.

(2) The development of the distinct morphological forms is facultative, being controlled largely by the environment, and in the case of the Collinsiella phase by the microhabitat. Although a trimorphic diphasis life history has not been previously reported for the Genus Enteromorpha, a link between morphology and environment has been recorded for Stigeoclonium species. "Only at a particular time of the year and under certain conditions does a plant reach its full size or 'peak' development .....". Klebs (1896), Godward (1942), Blum (1956) and others in their year-round investigations found that a particular species grows luxuriantly at a particular place or time of the year, whereas in other seasons it exists in a reduced growth-form, or disappears from the habitat (Islam, 1963).

A detailed description of the methods of reproduction in E. intestinalis has been presented in the preceeding section - 'Processes leading up to zygote and zoospore formation'. They may be summarised here for completeness. While the trimorphic diphasic life history of the Motunau population is maintained by a core of plants with a sequence of gamete and zoospore producing generations, these are augmented in the natural environment by the following means.

(1) Some plants morphologically intermediate between summer and winter forms produce both zoospores and gametes during the early winter months.

(2) Undischarged zoospores were able to germinate in situ into normal plants or formless anastomosing masses of filaments.

(3) Specialised vegetative cells could also develop into new plants by in situ germination thus omitting the motile reproductive stage.

(4) Detached vegetative fragments of the summer generation were liberated during early autumn in quantities alone sufficient to reestablish the species without any other means of reproduction.

(5) Either some entire winter plants or regions of gamete-producing plants were able to produce zoospores in early spring.

Few of these methods have been previously described and none in the detail of this thesis.

Discussion of the Taxonomic Implications of the Study.

The confirmation of the existence of branched and unbranched plants in the one population has important implications for the taxonomy of this species. Both simple and branched plants were included in Enteromorpha intestinalis (L) Link by Ahlner (1877). Bliding (1948) was able to establish the existence of sterility limits within one of these E. intestinalis - compressa populations, from which he concluded that the two halves constituted separate species. The simple half be referred to E. intestinalis, the branched one to Enteromorpha compressa.

However, Waern (1952) could not identify any indisputably simple threads - "they are always joined together at the base and thereby, according to Bliding already branched. However, I dare not identify the whole .... population with the compressa of Bliding without cross-

breeding experiments." Waern therefore described E. intestinalis as Ahlner (1877) had done. On the basis of the following evidence the present writer also retains Enteromorpha intestinalis sensu Ahlner (1877).

(1) Field observations linked the unbranched summer and branched winter population as separate generations of the same species.

(2) The branched winter plants gave rise to the characteristic summer generation under cultural conditions, thus confirming the conclusions drawn from field observations.

This study is the first to show the extent of a fund of non-genetically controlled variation as a normal part of the ontogeny of Enteromorpha species. Already several workers have shown that genetically controlled variations from the normal ontogenetic pattern may be used as taxonomic criteria. However, the writer demonstrated that the normal variation range of Enteromorpha intestinalis includes ontogenetic patterns taxonomically characteristic of Monostroma, Enteromorpha nana var minima (Yamada and Kanda, 1941) Blidingia, Percursaria, Enteromorpha hendayensis (Dangeard and Parriaud, 1960) and Collinsiella (Scagel, 1960). The proportion of many of these variants could be altered by variation of the cultural environment.

The results of the present investigation indicate that the genetically controlled variations from the normal may have originated as points on the non-genetically controlled variation range which have come under strict genetic control.

Summary of Life History of Enteromorpha intestinalis (L) Grev. from Motunau, North Canterbury.

(1) The population studied was found to have a facultative trimorphic diplohaplontic life history. The development of the distinctive summer and winter forms of morphology is controlled largely by the environment, that of the third Collinsiella form by the microhabitat.

(2) By a combination of observations in the natural environment and laboratory experiments, it was found that the majority of these plants have a regular alternation of winter gamete producing and summer zoospore producing generations with an accessory asexual (vegetative) cycle during early summer.

(3) The complexity of the reproductive cycle is increased by a range of additional methods, many previously unrecorded.

SUMMARY: STUDIES IN THE LIFE-HISTORY AND TAXONOMY OF THE GENUS ENTEROMORPHA

The Subject matter of this thesis may be summarised under the headings:-

(a) taxonomic character variation,

(b) culture methods,

and (c) the life history of Enteromorpha intestinalis.

TAXONOMIC CHARACTER VARIATION

1. The Ulvaceae is acknowledged by many algal systematists as a difficult group. Of all the populations sampled for this study, only that at Motunau could be confidently identified - as Enteromorpha intestinalis (L) Link sensu Ahlner 1877 p.15.

2. The writer postulated that most attempts at identification with information from existing records failed because of inadequate attention to the variation ranges of taxonomic characters. This was confirmed by the thorough investigation of the variation of the following characters in several populations: chloroplast morphology and orientation, chloroplast colour, presence (and number) or absence of pyrenoids, cell size, width of mucilage envelope, degree of convolution and its position on the thallus.

3. As a result of observations on freshly collected and artificially cultured plants, the writer recorded for the first time

(a) an ontogenetic cycle of change in the chloroplast morphology of Enteromorpha intestinalis,

and (b) a range of variation of this character in one species which included several distinct forms of chloroplast utilized in

the literature as taxonomic criteria for several different species.

4. The only character found to be sufficiently constant to be used as a major divisor within the Genus was cell diameter.

CULTURE METHODS

1. For short-term non-sterile cultures a peat or soil Erdschreiber solution was found most satisfactory.

2. For long periods of culture the writer developed a technique based upon a close approximation of conditions in the natural environment.

THE LIFE HISTORY OF ENTEROMORPHA INTESTINALIS (L) GREV.

1. Classification of the type of Life History. The life history of the population of this species studied at Motunau consists of a sequence of small finely branched winter gamete-producing and large simple summer zoospore-producing plants, with a well developed accessory reproductive cycle by mito-zoospores early in summer.

2. In addition to these two morphological phases a third Collinsiella phase was grown in artificial culture. This report of a trimorphic diplohaplontic life history for the Motunau population is the first life history other than monomorphic to be recorded for the Genus. There was evidence that certain stages in this life history are largely controlled by the environment.

3. Additional Methods of Reproduction. A range of reproductive means in addition to summer zoospores and mitospores, and winter gametes was found. Summer plants could produce gametes in autumn and early winter,

or give rise to the next generation by in situ germination of undischarged zoospores, specialised vegetative cells or fragments of vegetative tissue. On occasions in situ germination led to the development of formless anastomosing masses of filaments. The winter generation could also reproduce by zoospores or in situ germination of unreleased gametes.

4. Embryological Variation. An extensive range of non-genetically controlled variation in embryology was recorded for the first time as a normal feature of an Enteromorpha population.

5. Zooid Shape. A previously unrecorded range of zooid shape was found both in nature and in culture. This, together with observations of sporangial cleavage, support the conclusions that sporogenesis and to a lesser extent gametogenesis, are not nearly as regular as existing records suggest.

6. Zooid Release Stimuli. Several methods of initiating zooid release detailed in the literature were unsuccessfully tested on E. intestinalis. However, at certain times of the year only, zooid release proceeded without the application of precise stimuli, as often advocated.

7. Zoospore Release. Two methods of zoospore release were observed, one occurring in the natural environment, the other restricted to plants grown in culture. This method has not been previously recorded.

APPENDIX I

STATISTICAL ANALYSIS

The object of this analysis was to determine whether there was any relationship between plant size and chloroplast morphology. If, for example, a significant percentage of the smaller plants were dominated by a dark green homogeneous or stellate chloroplast this might have proved a useful taxonomic criterion.

The following method was adopted from CHAMBERS (1963). The range of height in the population was divided into six classes and a table set out showing the number of plants with each of the sixteen chloroplast types. The Chi square value was 37 for 60 degrees of freedom. This distribution was not considered significantly different from that expected by chance at the 0.05 probability level.

It was therefore concluded that there was no correspondence between any type of chloroplast and a particular size of plant in the Bluff population.

ACKNOWLEDGEMENTS

The writer wishes to thank Mr. F. McGregor, A.R.P.S. for assistance in the reproduction of the lined drawings, and Dr. E.J. Batham, Marine Biological Station, Portobello for fertile material from a local Enteromorpha population. The writer is also indebted to Miss M.A. Guthrie for typing this thesis and assisting with the organisation of illustrations.

The writer is indebted to Professor W.P. Philipson and the staff of the Botany Department, University of Canterbury for their constant interest in this study.

Finally, the writer wishes to thank Dr. Margaret Mayer without whose guidance and encouragement the success of this thesis would not have been possible.

BIBLIOGRAPHY

GARDH, J. 1883. Till. algernes systematik Vl. Aeta. univ. Lund 191 - 182.

LGEUS, Sven. 1951. Studies on the Cultivation of Algae in Artificial Light. Physiol. Plantarum. 4(4) 742 - 753.

LLEN, E.J. 1914. On the culture of the plankton diatom Thalassiosira gravida Cleve in artificial sea water. Jour. mar. biol. assoc. Vol. 10. Plymouth.

RASAKI, S. 1946. Studies on the swarmers and their development in Ulvaceae and Monostromaceae. Seibutu V. 1. No. 5 - 6.

RASAKI, S. 1953. An experimental note on the influence of light on the development of spores of algae. Bull. Jap. Soc. Sci. Fish. 19(4) 466 - 470.

RASAKI and SHIHIRA. 1959. Variability of Morphological Structure and mode of reproduction in Enteromorpha Linza. Jap. J. Bot. 17(1): 92 - 100.

AUDRIMONT, R. 1960(a). A propos de l'heliotactisme des zoospores d' Enteromorpha Linza (L). J. Ag. - Soc. Linn de Bordeaux 98.

AUDRIMONT, R. 1960(b). Influence d'un milieu riche en amino acides sur les premiers stades du developpement d' Enteromorpha Linza (L) J. Ag. Le Botaniste Series XLIII Fasc I-VI: 157 - 167.

LIDING, C. 1933. Uber Sexualitat und Entwicklung Bei der Gattung Enteromorpha. Svensk Botanisk Tidskrift. 27.

LIDING, C. 1939. Studien uber Entwicklung und Systematik in der Gattung Enteromorpha 11. Bot. notiser. Lund.

LIDING, C. 1944. Zur systematik der Schwedischen Enteromorphen. Bot. notiser.

LIDING, C. 1948. Uber Enteromorpha intestinalis und compress. Bot. notiser. 2. 123.

LIDING, C. 1955. Enteromorpha intermedia. A New species from the coasts of Sweden, England and Norway. Bot. Not. 180:2, 253 - 262.

LIDING, C. 1960. A preliminary report on some new Mediterranean Green Alvae. Bot. Notiser. Lund.

LIDING, C. 1963. A critical survey of European Taxa in Ulvales. Part 1. publ. Opera Botanica.

BLUM, J.L. 1956. The Ecology of River Algae. Bot. Rev., 22(5): 291 - 341.

BOLD, H.C. 1942. The Cultivation of Algae. Botanical Review 8, 69.

BOUILHAC, R. 1897. Sur la culture de Nostoc punctiforme en presence de glucose. C.R. Acad. Sci., Paris, 125: 880.

BOUILHAC, R. 1898. Presence de chlorophylle dans un Nostoc cultive a l'abri de la lumiere. C.R. Acad. Sci., Paris, 127: 119.

BROWN, L.B. 1915. Experiment with marine algae in fresh water. Publ. Puget Sound Biol. Station. 1:31 - 34.

BURROWS, Elsie M. 1958 a. Growth Form and Environment in Enteromorpha. Journal of the Linnean Society of London. A Symposium; Experimental Approaches to the Problems of Growth and Form in Plants.

BURROWS, Elsie M. 1958 b. Sub-littoral algal population in Port Euri Bay, Isle of Man. J. Mar. Biol. Assoc. U.K., 37: 695 - 700.

CARTER, N. 1926. An investigation into the Cytology and Biology of the Ulvaceae. Ann. Bot. 40: 665 - 691.

CARTER, N. 1932. A comparative study of the alga flora of two salt marshes I. Journ. Ecol. 20. 341 - 70. 1932.

CAURO 1958.

CHAPMAN, V.J. 1953. Report of the 7th Science Congress. Royal Soc. N.Z.

CHAPMAN, V.J. 1954. The Classification of the Ulvales and Siphonocladales. 8th International Congress of Botany 17: 93.

CHAPMAN, V.J. 1956. The Marine Algae of New Zealand. Part 1. Myxophyceae and Chlorophyclae. The Journal of the Lumiean Soc. of London. 360: 333 - 501.

CHAPMAN, V.J. 1957. Marine Algal Ecology. Botanical Review, 23.

CHAPMAN, V.J. 1960. Salt Marshes and Salt Deserts of the World. Plant Science Monograph.

CHAPMAN, V.J. 1961. The Marine Algae of Jamaica. Part 1. Myxophyceae and Chlorophyceae. Inst. of Jamaica Publ.

CHAPMAN, V.J. 1964. The Chlorophyceae. Oceanography and Marine Biology 2. Ed. H. Barnes.

CLAUSEN, J.; KECK, D.D. and HIESEY, W.M. 1939. The Concept of Species Based on Experiment. Am. Jour. Bot. 26: 103 - 106.

CORPELAN, L.J. 1964. Effects of Salinity on Algal Distribution. Ecology 45: 70 - 77.

DANGEARD, P. 1960. L'Enteromorpha Linza (Linne) d. Ag., Le Botaniste Series VLIII Fasc. I-VI: 103 - 117.

DANGEARD, P. et PARRIAUD, H. 1960. Sur une Enteromorphe nouvelle (E. hendayensis nov. spec.) a developpement du type tubulosa. C.R. Acad. Sci. 250.

DARLINGTON and La COUR. 1962. The Handling of Chromosomes.

DAVIS and HEYWOOD. 1963. Principles of Angiosperm Taxonomy. Publ. Oliver and Boyd.

DELF, E. Marion. 1912. The Attaching discs of the Ulvaceae. Annals. Bot. 26 403 - 408.

de VALERA, Mairin. 1940. Note on the difference in Growth of Enteromorpha Species in Various Culture Media. Eysiogr. Sallsk. Forhandl, Botio. Lund 1940.

DOBZHANSKY, Th. 1941. Genetics and the Origin of Species. Publ. Columbia University Press.

DODEL, A. 1876. Ulothrix zonata ihre geschlechfliche und ungeschlechtliche. Fortpflanzung Jahrb. f. wiss. Botanik. v. 1876.

DOTY and NEUHOUSE. 1954. The Distribution of Marine Algal in Estuarine Waters.

DREW, K.M. 1955. Life Histories in the Algae. Biol. Rev. Cambridge Phil. Soc. 30(4) 343 - 390.

DROOP, M.R. 1959. Chemical and Ecological Considerations in the Design of Synthetic Culture Media for Marine Algae. Int. Congress Bot. IX Recent Advances in Botany, Vol. 1.

DROOP, M.R. 1962. Organic Micronutrients in Physiology and Biochemistry of Algae. 141 - 156. Ed. R.A. Lewin.

FELDMANN, J. Ecology of Marine Algae in Manual of Phycology. Ed. G.M. Smith.

FELDMANN, J. 1952. Les Cycles de reproduction des Algnes et leurs rapports avec la phylogenie. Rev. Cytol. et Biol. Veg. 13: 1 - 49.

FØYN, B. 1955. Specific difference between Northern and Southern European populations of the Green alga Ulva lactuca L. Pub. della Staz. Zoo. di Napoli 27: 261 (1955).

FØYN, B. 1961. Globose, a Recessive Mutant in Ulva mutabilis. Botanica Marina III Fasc 2. 1961.

FØYN, B. 1962. Two Linked Autosomal Genes in Ulva mutabilis. Botanica marina IV Fasc. 1/2 1962.

FRITSCH, F.E. 1927. The Cultivation of Algae in "British Fresh Water Algae". 16 - 18.

FRITSCH, F.E. 1935, 1961. The Structure and Reproduction of the Algae. Vol. 1.

GAYRAL, P. 1960. Premieres observations et reflexions sur des Ulvacees en culture. Botaniste 43. Bordeaux.

GAYRAL, P. 1961 b. Description, reproduction et developpement. E. hendayensis Var salen sis Nov. var Botaniste Vol. 44.

GODWARD, M.B. 1942. The Life-cycle of Stipeoclonium amoenym Kutz. New Phytol., 41: 293 - 301.

GODWARD, M.B.E. 1956. Cytotaxonomy of Spirogyra S. subechinata and S. britannica. Jour. Linn. Soc. Lon. Bot. No. 361.

GURR, G.T. 1957. Biological Staining Methods. VI Edition, 1957.

HEDGPETH, J.W. 1957. Estuaries and Lagoons in Treatise on Marine Ecology and Palaeoeology.

HARTMANN, M. 1929. Untersuchungen uber die Sexualitat und Entwicklung von Algen III. Uber die Sexualitat und den Generationswechsel von Chaetomorpha und Enteromorpha. Ber. dtsch. bot. Ges. 47, 485.

HARVEY, H.W. 1933. On the rate of diatom growth. Journ. Mar. biol. assoc. Vol. 19 Plymouth 1933.

HENCKEL, A.H. 1926. Algologische Notizen. Bull. Inst. Rech. Stat. Biol. Univ. Perm. 4. 429 - 33, 1926.

HUNTER, E.O. and The effect of Antibiotics on pure cultures of Algae. Am. Jour. Bot. 48: 179 - 185.

HUXLEY, J.S. 1940. Ed. The New Systematics Oxford, Clarendon Press.

ISLAM, A.K.M. Nurul. 1963. A Revision of the Genus Stigeoclonium.

KLEBS, G. 1896. Bedingungen der Fortpflanzung bei einigen Algen und Pilzen. Jena. 1896.

KORNMANN, P. 1956. Zur Morphologie und Entwicklung von Percursaria percursa. Helgd. Wiss Meeresuntersuch, 5.

KORNMANN, P. 1965. Ontogenie und Sebenszyklus der Ulotrichales in phylogenetischer Sicht. Phycologia 4(3) 1965 pp 163 - 172.

KYLIN, H. 1930. Uber Heterogamie bei Enteromorpha intestinalis. Ber: dtsch. bot. Ges. 48. 455.

KYLIN, H. 1931. Uber Heterogamic dia E. intestinalis. Ber. d. Deutsch. Bot. Gesellsch Bot. 48 H.10.

KYLIN, H. 1941. Biologische Analyse des Meerlvassers. Kgl. Fysio. Salliskapet 11 1941.

KYLIN, H. 1942. Influence of glucose, ascorbic acid and heteroauxin on the seedlings of Ulva and Enteromorpha. Kgl. Fysiograf. Sallskap. i. Lund. Forh. 12: 135 - 148.

KYLIN, H. 1943. Uber du Ernahrung von Ulva lactuca. Dgl. Fysiografiska Sallskapets i. Lund. Forhandlingar.

KYLIN, H. 1946. Uber den Zuwachis der Keimlinge von Ulva lactuca in Verschiedenen Nahrflussigheiten. Proc. Roy. Physiographic Soc. Lund. (Kgl. Fysiografiska Sallskapets i. Lund. Forhandlingar.) 45 - 56.

KYLIN, H. 1949. Die Chlorophyceen der schwedischen westkuste. Lunds. Univ. Arassker. N.f. Avd. 2. Bol. 45. 2. 1949.

LERSTON and VOTH. 1960. Experimental Control of Zooid discharge and Rhizoid formation in the Green Alga Enteromorpha. Botanical Gazette. 122. 1960 - 61.

LEVRING, T. 1946. Some culture experiments with Ulva and artificial sea water. Kgl. Fysiograf. Sallskap. i. Lund. Forh. 16: 45 - 56.

LEVRING, T. 1955. Some remarks on the structure of the gametes and the reproduction of Ulva lactuca. Bot. Not. Lund.

LOVLIE, Arne. 1964. Genetic control of Division Rate and Morphogenesis in Ulva mutabilis. C.R. Des Travaux du Laboratoire Carlsberg. Publ. in Copenhague by Danish Science Press Ltd., 1964.

McFARLANE, C.I. 1959. Some Ecological problems encountered in Surveys of Marine Algae in Nova Scotia. Int. Congr. of Botany IX 1959. Recent Advances in Botany, Vol. 1.

MAYR, E. 1942. Systematics and the Origin of species. New Yord. Columbia University Press.

MOEWUS, H. 1938. Die Sexualitat und der Generationsweckel der Ulvaceen und Untersuchungen uber die Parthenogenese der Gameten. Arch. f. Protistenk., 91: 357.

MOEWUS, F. 1948. Zur Genetik und Physiologie der Kern - und Zellteilung 1. Die Apomiktosis von Enteromorpha Gameten. Biol. Zentr. 67: 277 - 293.

NAYLOR, M. 1954. A checklist of the Marine Algae of the Dunedin District. Trans. & Proc. Roy. Soc. N.Z.

OKUJAMA, K. Ecological Study of Enteromorpha (I) E. nana var minima. Sjostedt. Bull. Tohaku Reg. Fish. Res. Lab. 13: 108 - 114.

PAPENFUSS, G.F. 1950. Culturing of Marine Algal in Relation to Problems in Morphology. "The Culturing of Algae". Publ. by The Charles F. Kettering Foundation.

PAPENFUSS, G.F. 1960. On the Genera of the Ulvales and the Status of the Order. Journal of Linnean Soc. London.

PEACH, E.A. and DRUMMOND, J.C. On the culture of the marine diatom Nitzschia closterium f. minut isoima in artificial sea water. Biochem. Journ. Vol. 18. 1924.

PRINGSHEIM, E.G. 1912. Die Kultur von Algen in Agar. Beitr. Biol. Pfl. 11, 305.

PRINGSHEIM, E.G. 1921. Algenkultur. Abderhaldens. Handb. biol. Arb. Meth. Abt. XI, Heft 2, P.402.

PRINGSHEIM, E.G. 1949. 'Pure Cultures of Algae, Their preparation and maintenance'. Cambridge University Press.

PROVASOLI, L. 1957. Effect of plant hormones on seaweeds. Biol. Bulletin 113: 321.

PROVASOLI, L. 1958. Effect of Plant hormones on Ulva. Biological Bulletin 114: 375 - 384.

PROVASOLI, L. 1958 a. Effects of Plant Hormones on Ulva. Biol. Bull. Woods Hole 114: 375 - 384.

PROVASOLI, L. 1959. Growth Factor Requirements of Algae and Ecological Considerations. Int. Congr. of Bot. IX. Recent Advances in Botany Vol. 1.

RAMANATHAN, K.R. 1939. The Morphology, Cytology and alternation of generations in Enteromorpha compressa (L) Grev. var. lingulata. Annals of Bot. London. M.S. 3 375 - 98.

REYNOLDS, N. 1950. Methods of Culturing Epiphytic Algae. New Phytol. 49 July 1950 2. pp 155 - 163.

SCAGEL, R.F. 1964. Life History Studies of the Pacific Coast Marine Alga Collinsiella Tuberculaga Setchell and Gardner. Canadian Journal of Botany 1964, 38 969 - 983.

SCHILLER, J. 1907. Beitrage zur Kenntnis der Entwicklung der Gattung Ulva. Sitzber. Akad. Wiss. Wien. Mat.-nat. Kl. 1. 116 1691 - 1716. 1907.

SCHREIBER, E. 1935. Uber Kultur und Geschlectisbestimmung von Dictyota dichotoma. Planta. Bot. 24. Berlin 1935.

SETCHELL, W.A. and GARDNER N.S. 1920. The Marine Algae of the Pacific Coast. University of California. Publication Botany 8 139 - 374.

SINNOTT. 1960. Morphogenesis in Plants.

SMITH, G.M. 1938, 1964. Cryptogamic Botany.

SMITH, G.M. 1944. Marine Algae of the Monterey Peninsula.

SMITH, G.M. 1947. On the Reproduction of Some Pacific Coast species of Ulva. Amer. Jour. Bot. Vol. 34:2: 80 - 87.

STEBBINS, J.L. 1950. Variation and Evolution in Plants.

SUNESON, S. 1942. Uber wachstumsfordernde Wirkung von Algenextrakten auf Ulva and Enteromorpha. Kgl. Fysiograf. Sallskpt. i. lund. Forh.

SUNESON, S. 1943. Weitere untersuchungen uber wachstumsfordernde Wirkung Algenextrakten auf Ulva lactuca. Kgl. Fysiograf. Sallskpt. i. Lund. Forh.

SUTO, S. 1950. Studies on Shedding, Swimming and fixing of spores of Seaweeds. Bull. Jap. Soc. Sci. Fish. 16(1): 1 - 9.

TAYLOR, W.R. 1962. Marine Algae of the Northeastern Coast of North America.

TREBOUX, O. 1905. Organische Sauren als Kohlenstoffauelle bei Algen. Ber. dtsch. bot. Ges. 23. 432.

VISCHER, W. Uber primitivste Landpflanzen. Ber. Schwetz. bot. Ges. 63. 169 - 193, 1953.

WAERN, M. 1952. Rocky Shore Algae. Acta Phytogeogr. Sueciea. 30 Uppsala.

WARDLAW, C.W. 1964. Organisation and Evolution in Plants.

WEEKS, D.C. Preliminary Observations on Effects of Varying Salinity on Enteromorpha intestinalis. Reprinted from Rep. Challenger Soc. No.XIII, 1961.

WETTSTEIN, F.V. 1921. Zur Bedautung und Technik der Reinkultur dur Systematik und Floristik der Algen. Ost. bot. Z. 70:23.

WILLIAMS, J.S. 1905. Ann. Bot. 19: 531 - 560.

WOLKEN, J.J. The Structure of the Chloroplast. Ann. Rev. Pl. Physiol. 10: 71 - 86.

MERSLEY, H.B.S. 1956. A Critical Survey of the Marine Algae of Southern Australia.
Australian Journal of Marine and Freshwater Research 7:3 pp 343 - 383.

MERSLEY, H.B.S. 1956 b. The Marine Algae of Kangaroo Is. IV.
Aust. Jour. Mar. and Freshwater Res. 7(1): 64 - 87.

MADA, Y. and KANDA, T. 1941. On the Culture Experiment of Monstroma zostericola and Enteromorpha nana var minima.
Sci. Papers of Inst. of Algological Res. Fac. of Sci., Hokkaido Imp. Univ., Vol. II 2, 1941.

MADO, Y. and SAITO, E. 1938. On some culture experiments with the swarmers of certain species belonging to the Ulvaceae.
Sci. Papers of Inst. Algol. Res. Fac. of Sci., Hokkaido Imp. Univ. Vol. II. No. 1, 1938.

MADA, Y. and SAITO, E. 1939. On some culture experiments with the swarmers of certain species belonging to the Ulvaceae.
Fac. of Sci. Hokkaido Imp. Univ., 2. 35, 1939.